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CONTENTS

The Biology and Control of the Camel Cricket *Daihinia brevipes*.....F. E. WHITEHEAD AND FLOYD D. MINER 573
The Life History of *Phyllophaga calceata* and *P. micans*.....H. J. REINHARD 581
Relation Between Host Condition and Attacks by the Bronzed Birch Borer....ROGER F. ANDERSON 588
Effect of Soils, Cover Crops and Foliage on Concentration of HCN in Citrus Fumigation.....ROBERT A. FULTON AND R. L. BUSBEY 597
Wheat Flour as an Adhesive for Sprays.....LEWIS T. GRAHAM, R. M. SANDSTEDT AND H. DOUGLAS TATE 599
The Protective Value of Asphalt Laminated Paper against Certain Insects....HARVEY L. SWEETMAN AND A. I. BOURNE 605
The Use of Oleic Acid and Aluminum Sulfate to Increase Deposits of Nicotine Bentonite.....C. C. ALEXANDER, C. C. CASSIL, F. P. DEAN AND E. J. NEWCOMER 610
Repellency of Pyrethrum Extract and Other Materials to Full-Grown Codling Moth Larvae.....M. A. YOTHERS AND F. W. CARLSON 617
A Dust Experiment for Codling Moth Control in a Heavy Infestation.....MARTIN M. BARNES 620
Effect of Scraping and Banding Trees upon the Numbers of Transforming and Hibernating Codling Moth Larvae.....HOWARD BAKER 624
Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity as Fumigants.....H. L. KING AND D. E. H. FREAR 629
Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity against *Tribolium confusum*.....H. L. KING AND D. E. H. FREAR 634
Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity to *Aphis rumicis*.....H. L. KING, D. E. H. FREAR AND L. E. DILLS 637
Mercury Substitutes for Cabbage Maggot Control.....L. E. DILLS, D. E. H. FREAR AND H. L. KING 640
Strength of Rotenone Dust Mixtures and Rate of Application in Pea Aphid Control.....J. E. DUDLEY, JR., AND T. E. BRONSON 643
Laboratory Technique for Testing Insecticidal Dusts for Pea Aphid Control.....C. E. DIETER, R. L. JANES, H. F. WILSON AND H. L. BURDICK 646
Electrostatic Charge Effects Produced by Insecticidal Dusts.....H. F. WILSON, R. L. JANES AND E. J. CAMPAU 651
Toxicity of Cryolite to Mexican Bean Beetle Larvae.....GEORGE WENE AND ROY HANSBERRY 656
Heliothis virescens as a Pest of Cotton with Notes on Host Plants in Peru.....EDSON J. HAMBLETON 660
Comparative Toxicities of Copper Hydroarsenate and of Copper Hydroarsenate-arsenite Mixtures.....JAMES W. APPLE AND CHARLES H. RICHARDSON 666
Further Tests of Synthetic Organic Compounds as Insecticides.....M. C. SWINGLE, E. L. MAYER AND J. B. GAHAN 672
The Repellency of a Pyrethrum-Thiocyanate Oil Spray to Flies attacking Cattle.....D. E. HOWELL AND F. A. FENTON 677
Observations on the Biology of the Stablefly in Florida.....S. W. SIMMONS 680
Screwworm Survey of the Southeastern States in 1943.....W. G. BRUCE 687

The Toxicity of Sulfur Dioxide, Acetone and Ethylene Oxide alone and in Combination ..... Ely M. Swisher 690
Sulfur Dioxide-Acetone as a Household Fumigant ..... Ely M. Swisher 694
Effect of *Empoasca solana* on Sugarbeets Grown for Seed ..... Orin A. Hills, Van E. Romney and K. B. McKinney 698
Susceptibility of Japanese Beetle Larvae to *Bacillus popilliae* ..... Raimon L. Beard 702

Scientific Notes

Difference in Damage by Three Species of Lygus to Alfalfa Seed Production .. Loyd L. Stitt 709
Tests of Poisoned Baits to Control the Tobacco Budworm ..... J. M. Grayson 709
Ether-Extract Content of Codling Moth Cocoons ..... F. W. Carlson, C. C. Cassil, and M. A. Yothers 711
Olfactory Responses of Parasitic Hymenoptera in Relation to their Mass Production ..... Stanley E. Flanders 711
Two Important Parasites of the Tobacco Budworm ..... J. M. Grayson 712
A New Weed Host of the Beet Leafhopper .. J. R. Douglass, H. C. Hallock, and W. E. Peay 714
Two Introduced Insects Attacking the Woolly Apple Aphid in California ..... A. E. Michelbacher and A. D. Borden 715
The Power of the Analysis of Variance with the Poison Distribution ..... Wm. M. Upholt 717

Editorial: Taking Stock ..... E. O. Essig 718

Obituary: Harrison Garman 721

—NOTICE TO SUBSCRIBERS—

—Copies of February Journal Needed—

The unprecedented demand for the articles on DDT absorbed all of the separates and before we knew it all the extra copies of the Journal. We must have 35 copies of the February Journal, Volume 37, No. 1 to complete a standing order for libraries. It occurs to the writer that some subscribers might be willing to sell us their copy of the February 1944 Journal possibly after the close of the year or after they have read and used it for reference during the year. We will be glad to pay 75¢ per copy for any that can be spared or to credit the amount on the individual's 1945 subscription.

NOTICE TO MEMBERS

Professor Ray Hutson, Chairman of the Committee on Common Names of Insects, urges that any members who have proposals for new common names for any insects, or for changes of any of the approved names, submit their proposals to him as soon as possible so that they may be included in the next list issued for consideration of the membership. Address, Professor Ray Hutson, East Lansing, Mich.

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The Biology and Control of the Camel Cricket *Daihinia brevipes*

F. E. WHITEHEAD and FLOYD D. MINER,[1] *Oklahoma A. & M. College, Stillwater*

A camel cricket, *Daihinia brevipes* Haldeman, was on numerous occasions reported to be injuring a wide range of crops in the seedling stage in Oklahoma during the years 1935 to 1938. The reported injury consisted chiefly in the cutting off of seedling plants near the ground in a manner similar to that of cutworms. Almost nothing was known of the life history or economic importance of this insect, a surprising situation considering its large size and the large numbers present in certain areas of the state. The species was found to be common over a large part of Western Oklahoma. While serious injury to crops did not occur during 1939 and 1940 when the insect was studied, there seems to be little doubt that it is capable of doing considerable injury to a variety of crops.

A study of the unusual life history and habits of this species, several aspects of its biology and its insecticidal control was made by the Department of Entomology, Oklahoma Agricultural Experiment Station, during 1939 and 1940.[2] Intermittent observations also were made both before and after this two-year period during which intensive work was carried on. The experiments were conducted primarily at Crescent, Oklahoma, although some data were taken at other localities in the state.

REVIEW OF LITERATURE.—Haldeman (1850) described the insect as *Phalangopsis brevipes*, creating at the same time a new subgenus *Daihinia* for the new species. *Daihinia* was raised to generic rank by Scudder (1862). Since the papers of Haldeman and Scudder there are very few references to this species. Caudell (1916) briefly reviewed this genus stating that *D. brevipes* occurs from Louisiana north to Wyoming and North Dakota. Hubbel & Ortenburger (1927) listed *D. brevipes* as occurring in Oklahoma. A note by Hyslop (1934) states that the species was reported as injurious to garden and truck crops at Hollis, Oklahoma. This is the only place in literature where it has been recorded as being injurious. Hebard (1936, 1938) and Blair & Hubbel (1938) also list the species as occurring in Oklahoma. Hubbel (1936) published "A Monographic Revision of the Genus Ceuthophilus" in which is found biologic references to *D. brevipes*. He also records *D. brevipes* as being found in the Great Plains States from North Dakota to northern Texas.

DISTRIBUTION IN OKLAHOMA.—*Daihinia brevipes* is found almost throughout the sand hills areas of western Oklahoma; the only known exception is the region near Enid in Garfield County, where the species has not yet been found. Occasional individuals have been collected outside of these sandy areas, but never in large numbers. The area over which the crickets are potential pests includes approximately one-fifth of the western half of the state.

DESCRIPTION.—The body of the adult is thick, robust, and roundly humped dorsally (Fig. 1). The color varies from brownish-red to dark brown, generally a little darker on the dorsum of the thorax except for a narrow median line of clay yellow. A mottling of lighter shades occurs on nearly all parts of the body. All the tibiae are well provided with spines; the anterior tibiae are semi-fossorial. The posterior femora of the male are remarkably heavy, and from five to seven heavy spines occur on the apical half of the outer edge.

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[2] The authors wish to express appreciation for the assistance of John Standish and Howard Eckley, students in the Department of Entomology, Oklahoma A. & M. College, who collected some of the data used in this report.

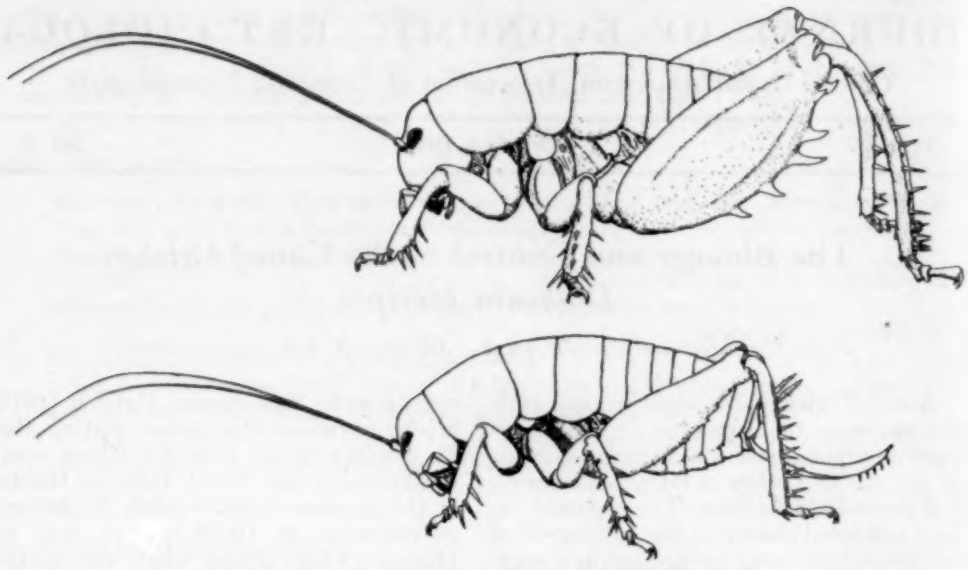

FIG. 1.—*Daihinia brevipes* Haldeman (above, male; below, female).

The armed posterior femur and tibia serve prehensile purposes in combats frequently indulged in by the males; kicking is the chief method of attack, however. The anterior and posterior tarsi are three segmented, the medial ones four segmented. The body measures from 20 to 28 mm. in length.

The egg is about 2 mm. long, and in shape is broadly oval. One end is slightly broader than the other. When first deposited the color is creamy white, changing to a dark tan as incubation progresses. The chorion is marked with microscopic reticulations.

The first of the six nymphal instars is about 6 mm. in length and tan in color. The succeeding instars undergo a gradual increase in length and darkening of color, but in general there are no marked structural differences between instars. The characters most readily used in differentiating instars are the length of the cerci of the male and the length of the ovipositor of the female.

SEASONAL HISTORY AND BIOLOGY.—The seasonal cycle was determined chiefly by collecting the insects at frequent intervals throughout the year. This information was supplemented by rearing individuals in cages constructed of one-gallon tin cans with a three-inch circular hole cut in the bottom, sunk in sandy soil to a depth of 3 to 4 inches (Fig. 2). The insects placed

FIG. 2. Above—Field trap for camel crickets. Below—Breeding cages used in studies.

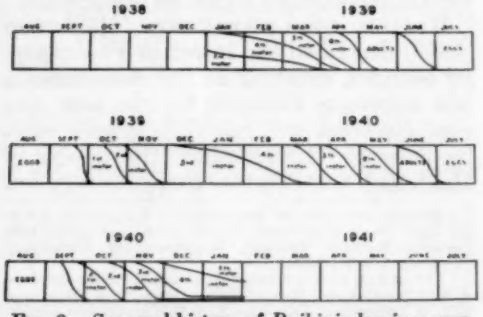

FIG. 3.—Seasonal history of *Daihinia brevipes* over a period of 25 months.

in such cages burrowed into the soil through the hole in the bottom of the can and could then be trapped at will for examination by placing a screen-lined metal cylinder over the burrow. The screen lining enabled them to crawl up the cylinder and fall to the outer part of the can, but the smooth outer surface of the cylinder prevented their re-entering the burrow until the cylinder was removed. The spines of the posterior tibiae were kept marked with red finger nail polish, the disappearance of which indicated that a molt had been undergone. Moistened bran and various kinds of green plant material were kept in the cages as food.

Records of egg deposition and incubation were obtained by sifting the soil surrounding burrows in the field through an 18-mesh copper screen. Approximate dates of hatching were indicated by discovery of nymphs or remnants of egg shells.

The seasonal history is shown in Fig. 3. There was considerable variation in seasonal development but it may be generalized as follows: the winter is passed in burrows primarily as third and fourth instars although a few second or fifth instars may be present. Occasional activity throughout the winter is indicated by the presence of fresh soil at the entrances of burrows on warm days; when dug from their tunnels the insects are always active although somewhat sluggish on colder days. There is only slow development throughout the winter, but during March rapid development begins and by the latter part of April or early May adults appear. Egg deposition in the walls of the burrows begins during the latter part of May and continues until the first of July, by which time most of the adults have died. The eggs hatch during the latter part of September. Since the burrows constructed by the adults have been obliterated by the time the eggs hatch, the nymphs must dig their way to the surface; eggs were frequently found at a depth of 4 or 5 feet. Upon reaching the surface the nymphs dig individual burrows. Development continues until cold weather arrives, when most of them have reached the third or fourth instar. There is one generation a year.

It is thought that copulation usually occurs within the burrow since it was observed on the surface on only one occasion. The fact that after maturity is

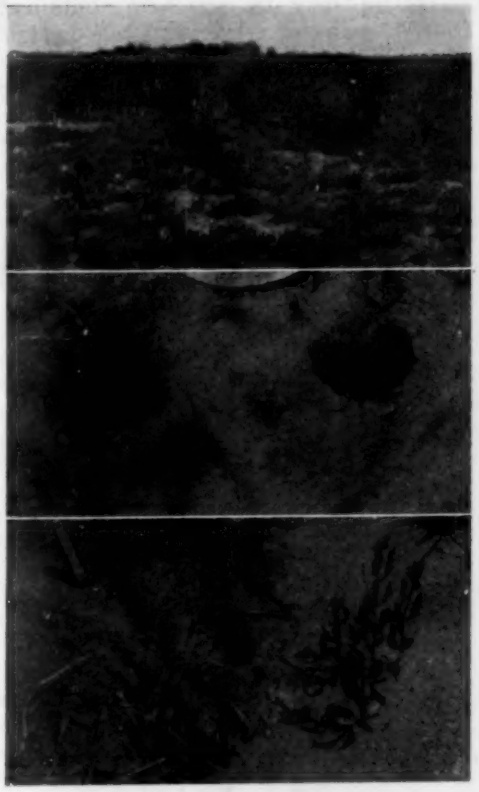

FIG. 4.—Above: Plot used in studies; white stakes indicate individual burrows. Center:—Burrow plugged at entrance on left. Ant burrow on right. Below—Appearance of burrow when plugged beneath the surface.

reached several males and females habitually occupy the same burrow is additional evidence for this supposition.

Normal egg deposition was of course impossible to observe. Sifting of soil surrounding burrows indicated that the eggs are inserted singly in the walls of the tunnel, the majority probably in branches off the main tunnel. Two eggs were excavated undisturbed in one of these branches. In shallow burrows of two feet or less in depth most of the eggs were found near the bottom; in burrows of 4 to 5 feet in depth, however, the distribution was rather uniform from a depth of about two feet to the bottom of the burrow. Clay subsoil was the common factor limiting depth of the burrow.

In an examination of 25 burrows an average of 26.8 eggs to a burrow were found with a maximum of 128. Attempts to induce oviposition in cages were not very

successful, only 18 eggs being obtained from 75 pairs of crickets so caged. It is probable that all the above figures are too low, since dissection of gravid females frequently showed as many as 30 nearly mature eggs to be present, with several times this number in a less advanced stage of development. Mature eggs were found in the ovaries as late as the last of June.

Incubation in the field requires about 3.5 to 4 months. Eggs incubated under conditions allowing observation failed to hatch except when collected in an advanced stage of development.

FIG. 5.—Casts of camel cricket burrows.

The process of molting was observed once in a glass walled cage, and a few camel crickets were dug from the soil while they were molting. Each observation indicated that the exuviae are habitually eaten. It was also found that at the time of molting the insect remains continuously in the burrow for a considerable length of time. In observation cages the average length of time spent continuously in the burrow when molting into the 4th, 5th, 6th, and adult instars was 19.6, 16.7, 10.8 and 10.0 days respectively.

BURROWING HABITS.—The procedure followed by the insects in digging a burrow was observed by confining individuals in vertical glass tubes filled with sandy soil. The forelegs are used first to loosen the soil; then, while the insect backs upward toward the burrow entrance, they are used to drag the loosened soil along under the body. This soil is deposited at the entrance, after which the cricket walks forward over it and kicks it outside by alternating quick strokes with its hind legs. Adults frequently kick soil to a distance of 18 inches from the entrance of the burrow.

A plug of soil is kept in the burrow while the insect is inside unless dry soil conditions make this impossible. In the spring the plug is at or near the entrance. Later when the surface soil is dry and the burrows larger, the mouth of the burrow is open and the plug is found farther down in the burrow (Fig. 4). This habit of keeping the burrows sealed no doubt offers some protection from natural enemies.

The depth and shape of the burrows was determined by first trapping all individuals from the burrows, so that no plugs would be present, and then pouring Portland cement of a thin consistency into the burrow. When the cement had hardened, the cast was dug out. Ordinary excavation was generally not practical for studying details of the burrow due to the loose sandy nature of the soil. Figure 5 shows a few typical concrete reproductions of burrows of adult crickets. The burrows were found to be 3 to 6 inches deep from October through February; in March they ranged from 3 to 12 inches in depth; in April and May the depth varied from 12 to 18 inches. In June most of the burrows ranged from 1 to 3 feet, although many burrows in deep sand were as deep as 4 or 5 feet.

Throughout the winter and until June the burrows are single shafts; shortly after the insects mature, however, branches are dug off the main shaft at various depths in the burrow. These branches have a diameter slightly less than that of the main shaft, are generally more nearly horizontal, and may extend for a foot or more away from the main tunnel. Occasionally these branches in turn have other short branches leading away from them. When clay subsoil is encountered the burrow generally turns sharply and follows the plane of the clay for a few inches.

FACTORS AFFECTING EMERGENCE FROM BURROWS.—Conditions influencing nocturnal emergence from burrows were studied because of the direct relationship of this information to timing applications of

poison bait being used in control experiments. Traps similar to the observation cages (See Fig. 2) but of smaller size were set daily over 50 freshly-worked burrows and the number of insects taken while attempting to leave their burrows was recorded. The percentage of emergence for the night was obtained by dividing the number of insects caught by the number of traps set. Results obtained by this method represent only the emergence from freshly-worked burrows and not emergence of the entire population. It is known that many camel crickets remain in their burrows for long periods of time during which the burrow appears abandoned or is obliterated by wind and rain.

Analysis of these daily records taken from February 24 to June 22, 1940, showed little correlation of percentage of emergence with either temperature or rainfall. Field observations in general agreed with these results. Camel crickets emerged in small numbers on nights when temperatures dropped below freezing; they were observed out feeding in large numbers on cold nights in late winter, and on one occasion in late February several were seen digging within a few inches of a snowdrift. Nightly temperatures were not known to have been sufficiently high to retard activity but individuals exposed to daytime temperatures during hot weather died within an hour or two. The average nightly emergence over the entire season as determined by trapping was 44.3 per cent; during the time when crop injury is most likely to occur, however, the emergence is definitely lower than this average.

A similar study of emergence habits was made with camel crickets confined in the previously described observation cages. In these cages, where the entire population could be accounted for, there was a positive correlation between emergence and temperature at 8.00 P.M. provided that insects remaining in their burrows for molting were disregarded in the analysis. Daily variation was great, however. Emergence of the population as a whole on any given night, as in the field experiment, was so variable that bait applications could not be timed by this method. Both of the above experiments indicated that emergence is much more frequent after the adult stage is reached.

The hours of emergence and greatest activity were studied by noting the number of camel crickets observed in the field during periods of 5 minutes each at intervals throughout the night, and by inspecting traps hourly during the night. These studies indicate that the majority of the insects emerge during the first 2 hours of darkness, after which the remainder come out in small numbers throughout the night. Near midnight they gradually begin returning to their burrows and by dawn only an occasional individual can be seen.

In previous years considerable numbers of camel crickets were frequently seen on the surface some time before sundown, but in 1940 they almost never left their burrows until it was practically dark. It was observed, however, that at or shortly before sundown they commonly begin digging within their burrows. At this time nearly all the active burrows are open and have fresh soil thrown out at the entrance.

BURROW RETENTION AND DISTANCE TRAVELED FROM BURROWS.—Habits with regard to retention of any one burrow and distances traveled when changing burrows were studied by trapping previously marked camel crickets, since individuals could not be directly observed in their nightly movements without disturbing them with a flashlight. In these studies a circular plot 90 feet in diameter was laid out in an alfalfa field. Each burrow within the plot was staked and numbered as it appeared and the insect in it trapped and marked by a system of clipping tibial spines, after which it was replaced in the burrow. After a sufficient time had elapsed for it to have emerged several times, the burrow was again trapped to determine if the same specimen still occupied the burrow. This periodic trapping of each burrow in the plot and marking of all unmarked individuals captured continued from March 15 to June 7, 1940. The results are summarized as follows:

Total captures recorded	765
Total captures of unmarked specimens	514
Number digging new burrows	386
Number in burrows dug by other individuals	128
Total captures of marked specimens	140
Number in same burrow as previously	92
(Days retained, average, 17.4; maximum, 42).	
Number in a different burrow	48
Number digging new burrows	31
(Distance to new burrow, average, 26.5 ft.; maximum, 72 ft.)	
Number in burrows dug by other individuals	17

(Distance to new burrow, average, 17.3 ft.; maximum, 54.3 ft.)
Total discarded (dead, injured, etc.)..... 111

These data indicate a definite tendency for camel crickets to retain the same burrow over a considerable period of time, presumably returning to it after feeding. When one abandons its original burrow it apparently digs a new one more often than it appropriates the burrow of another individual. Distances traveled are not indicative of any rapid, extensive migration of an infestation.

Experiments to determine distances traveled during nightly foraging were largely unsuccessful. The method used was to trap marked individuals in pitfalls set at varying distances from their burrows. Very few marked camel crickets were captured in this manner, although unmarked crickets were taken in abundance. The maximum distance any individual was taken from its burrow was 30 feet. Some indication of distances traveled while feeding was given by baiting experiments to be discussed later. In this work camel crickets from outside the plot limits were commonly killed, but no reduction in infestation was detected farther than 50 feet from baited areas.

NATURAL ENEMIES.—Parasitic and predaceous enemies which might account for an observed rapid decrease of populations were sought throughout the investigation. In both 1939 and 1940 the decline in field population was quite marked, and in 1940 only 18 of 105 fourth instar crickets placed in observation cages reached the adult stage, a survival of 17.2 per cent. The only indication of parasitism was obtained in 1939 when a dipterous pupa was found in a burrow with a dead female camel cricket. A mite, probably a symbiont, was taken from a preserved adult male and identified as a species of *Macrocheles*.[1] Possible predators were collected for stomach examinations during the spring and early summer of 1940, including the more common birds, rodents, and reptiles found in the Crescent community. Only two were found to contain remains of *Daihinia brevipes*, namely a ground squirrel, *Citellus tridecimlineatus*, and a sand swift, *Cnemidophorus sexlineatus*. Five specimens of the former contained the remains of four of the insects and in 29 specimens of the latter were found the remains of 6 camel crickets. During the spring of 1939 there was also considerable evidence that house cats were feeding on camel crickets confined in the observation cages.

Ground squirrels are believed to capture camel crickets by digging them out, as freshly-dug ground squirrel burrows were frequently observed to have followed a burrow downward. The sand swifts were often observed entering the burrows. They apparently were able to capture only those camel crickets in burrows without a plug of soil.

Known predators, however, would seem not to account for the high mortality known to occur. Hubbell (1927) has recorded several species of pathogens known to attack other members of this group of insects. Very little evidence of disease was encountered during this investigation and no pathological work was done.

FOOD AND INJURY.—Camel crickets have been reported by farmers to injure tomato, watermelon, cotton and cowpea seedlings. They have also been reported to be nuisances in lawns where their digging dries out the soil and disfigures the grounds.

Feeding habits were studied in 1939 and 1940 by field observation at night, by cage experiments, and by stomach examinations. It was found that an unusually wide variety of plant and animal material, both living and dead, is acceptable as food. Feeding was observed on growing cotton, alfalfa and roadside grasses, but never to the extent of economic injury. Other material on which the species was observed to feed included dry sticks, dead leaves, pecan nuts, rabbit pellets, bird droppings, carcasses of toads, and live ants of several species. Ants seem to be particularly desired as food. Often as many as seven or eight individuals could be seen after dark near an ant den, apparently lying in wait for the occasional ants which were out at this time.

When feeding on living plants the camel crickets remain on the ground, feeding on those leaves which can be reached or gnawing the stem of the plant until it falls of its own weight. After having cut off a plant the insect sometimes feeds further on the fallen plant and at other times leaves it at once.

Since it was possible that the 1939–40 population of about one burrow to 5 square yards was considerably less than

[1] Identified by H. E. Ewing, U. S. National Museum.

that of former years when injury had been reported, high populations were confined in large outdoor pens in an attempt to study plant injury. The results were unsatisfactory, however, since it was impossible to maintain a high infestation in these enclosures. Circumstantial evidence indicated that cannabalism was chiefly responsible. One pen which was illuminated at night in an attempt at observation of nocturnal habits gave ample demonstration of the insectivorous habits of the species. Scarabaeid beetles were attracted to the light and those which fell into the pen were at once viciously attacked and devoured.

The experiment which came nearest to producing the type of injury described by growers was conducted in a greenhouse in 1940 with a relatively small number of camel crickets. Four series of cages contained respectively the following available food material: young watermelon plants, cowpea seedlings, tomato plants and tomato plants along with a supply of freshly-moistened bran. None of the watermelons or cowpeas were cut off and only a slight amount of leaf feeding was recorded. In the cages containing tomatoes, however, a total of 64 plants were cut off by the nine camel crickets in these cages and of this number 55 were cut off by five of the insects in the cages supplied with moistened bran. While the sample was small it is a further indication that these insects may be capable of serious injury under certain conditions.

The stomach contents of 57 individuals collected in the field throughout the season were examined and the amount of several types of food present was estimated for each one in terms of per cent of the total volume. The average stomach content was as follows: green plant material, 18 per cent; other plant tissue thought to be from living plants, 6 per cent; dead plant tissue, 30 per cent; insect remains, 31 per cent; miscellaneous and unidentifiable material, 15 per cent. Insect remains included identifiable fragments of five individuals of *Daihinia brevipes*.

On several occasions small masses of plant material clipped to short lengths were found at the bottom of camel cricket burrows. It is possible this material had been stored as food to be used during a stay underground.

While serious injury to field crops was not observed, the habits of these insects suggest at least one crop situation in which injury might be expected if a large population were present. It is evident that the species has no consistent preference for growing plants. If, however, a high infestation should pass the winter in a field which in the spring was planted to some crop involving a relatively few seedlings and then clean cultivated, the crickets would be forced to feed largely on these seedlings. Because of their habit of feeding on stems, the proportion of plants completely destroyed certainly would tend to be large.

BAITING EXPERIMENTS.—Control experiments were confined entirely to baiting since this method appeared promising in early trials. A poisoned bait consisting of bran, sodium arsenite and water was spread in 1937 on a farm near Hennessey, Oklahoma, where the insects were said to be injuring tomatoes and watermelons. It was evident that both the population and injury were definitely reduced, although no technique had been developed for taking accurate data on results obtained. A variety of similar mixtures used in 1939 at Crescent reduced populations by from 46 to 92 per cent.

The possible value of molasses in such a bait was suggested by a statement by Hubbell (1936) that in collecting closely related insects good results are obtained by sinking pint fruit jars in the ground until the top is level with the ground and baiting them with molasses. Brief experiments in 1939 and 1940 with similar baited traps showed molasses to be sufficiently attractive to justify testing in actual control work.

Baiting in 1940 was aimed chiefly at confirming the dependability of a formula believed to be satisfactory rather than with development of the best possible formula, since the status of this insect as a consistent pest was unsettled. Two formulas were tested. These were: (1) wheat bran 100 lbs., sodium arsenite 2 qts., water, 12 gal. and (2) the same formula but with 1 gal. blackstrap molasses added. The rate was 15 lbs. dry weight per acre.

In conducting the tests three 0.10 acre plots 50 feet apart were used for each experiment; all active burrows up to 50 in number in each plot were marked with a stake. A bait was spread near sundown on each of two of the plots, the third being

reserved as a check. After the baits had been out for two nights all staked burrows were trapped for two successive nights, the kill being determined by dividing the number of specimens trapped on a baited plot by the number trapped on the check plot. Correction was made for any discrepancy in number of burrows trapped. Tabulation of all experiments conducted

erations must be conducted to effect economical control. Since camel crickets so frequently remain under ground for considerable periods of time, especially during the spring when damage is most often reported, it is estimated that the bait should be kept on the ground for a period of 12 to 15 days. Since it does not seem essential that the baits be fresh, two to four bait-

Table 1.—Summary of Baiting Experiments. Crescent, Oklahoma, 1940.

Experiment Number	Date	Check		Bait With Molasses		Bait Without Molasses		Percentage of Kill	
		Number Holes	Number Crickets	Number Holes	Number Crickets	Number Holes	Number Crickets	Bait with Molasses	Bait without Molasses
1	5–13	17	10	9	0	15	1	100	88.6
2	5–13	19	8	5	0	15	3	100	52.5
3	5–13	8	8	22	1	19	0	95.5	100
4	5–17	40	20	40	8	40	5	60.0	75.0
5	5–24	22	35	20	2	20	3	93.7	90.6
6	5–24	22	18	19	2	26	3	87.2	85.9
7	5–24	20	9	17	0	23	4	100	61.3
8	5–27	50	24	50	8	50	3	66.7	87.5
9	5–27	50	31	50	1	50	4	96.8	87.1
10	5–29	50	27	50	4	50	5	85.2	81.5
11	5–31	50	17	50	20	50	10	0.0	41.2
12	5–31	50	25	50	4	50	18	84.0	28.0
13	5–31	50	54	50	5	50	16	90.7	70.4
14	6–4	50	40	50	8	50	21	80.0	47.5
15	6–5	50	98	50	10	50	29	89.8	70.4
Average Kill.[1]								82.3	71.1

[1] Computed from original data—not individual percentages of kill.

by the above method appears in table 1.

Statistical analysis indicated that the figures representing kills were highly significant for both baits. The difference in kill between the two different baits, however, was not significant. It is possible that a large amount of additional data would demonstrate a slight advantage in favor of the molasses bait.

Some uncontrolled condition obviously was encountered in experiment 11. There is no known explanation.

It should be emphasized that the average kills of 71 and 82 per cent recorded in the table do not apply to the entire population in the plots but only to those crickets active during the baiting period. Undoubtedly many camel crickets did not emerge during this time.

Control experiments were not conducted specifically to determine how often and over what period of time baiting op-

ings during this period should be sufficient These applications should be made regardless of temperatures since heavy emergences apparently are unpredictable.

SUMMARY.—*Daihinia brevipes* Haldeman, a camel cricket found in the Great Plains states from North Dakota to northern Texas, has been reported from Oklahoma as injurious to a variety of crops, such as tomatoes, watermelons, cotton and cowpeas, while in the seedling stage. It feeds at night, spending the day in burrows in the soil. In Oklahoma it is found mostly in sandy environments. Serious crop injury was not observed during two years of intensive study, but the habits of the insect indicate it may become injurious under certain conditions.

Winter is passed in burrows as a semi-active nymph. The adult stage is reached about May 1 and oviposition occurs throughout June. The eggs, laid in the soil

surrounding the burrow, hatch in late September and the nymphs dig their way to the soil surface. There is one generation a year.

Nocturnal activity was not closely correlated with weather conditions. Migration appears to be very limited. The burrows increase in depth from 3 inches in February to 1 to 5 feet in June. An average of 26.8 eggs were found in the soil around each burrow.

The species is general in feeding habits. In addition to growing plants it was observed to feed on such material as dry sticks, weed seeds, rabbit pellets and other insects including its own species.

Poison bran mash bait gave satisfactory kills in control experiments.—4-3-44.

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The Life History of *Phyllophaga calceata* and of *P. micans*[1]

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Previous studies have shown that the life history of various species of *Phyllophaga* is generally less protracted under the milder climatic conditions in the South. Widely distributed species which complete the life cycle locally in one year may require two or three years for the development of a complete generation in the northern half of the country. The problem of control or combative measures against white grubs is therefore regional in nature and depends in large measure upon a knowledge of the biology of a given form under local conditions.

Studies designed to meet this need were begun in 1937 and continued through the past seven seasons. During the course of this work the local life history of a number of economic forms has been determined and reported in previous papers (Reinhard 1940, 1941, 1942). The present paper is a continuation of this series and presents the data recorded on rearings under controlled conditions of two additional species. The observations on *Phyllophaga calceata* (Leconte) extended through 1942 and 1943 and those on *P. micans* (Knoch) were begun in 1941 and continued throughout the two subsequent seasons.

Rearing Methods: Rearing white grubs from egg to adult in close confinement is not a simple procedure. One of the more important immediate problems considered in these studies, on which progress can be reported, relates to the food requirements of the developing grub. The high rate of mortality attendant, expecially in the case of very young grubs, when fed upon sprouted kernels of grain emphasized the need for finding a more adequate

[1] Contribution No. 830, Division of Entomology, Texas Agricultural Experiment Station.

larval food. In the course of the tests with various kinds of materials it was found that grapenuts filled the requirements for an apparently satisfactory larval diet. During the past five seasons twenty or more different species have been carried through larval development on a sole diet of grapenuts. Newly hatched grubs accept this food readily and make rapid growth, which is maintained throughout the subsequent stages. The adults secured from these rearings were normal, sexually matured beetles, which fed, mated and oviposited readily during the usual period for these activities.

Eggs were screened from the soil in oviposition cages at 3 or 4 day intervals and transferred in lots of 10 to 100 or more to 6-oz. tin boxes one-half full of moist sterilized sand. The boxes were kept in a rearing cellar where incubation proceeded under temperature conditions averaging about 65° F. Daily inspections were made and all infertile or injured eggs were removed to prevent growth of any molds. A day or two prior to the time of hatching the eggs were removed to new boxes containing moist firmly packed soil with a series of small impressions or cavities in the surface. One egg was placed in each of the latter to prevent the newly hatched grubs from coming into contact and injuring each other. Larvae hatching in cells not exceeding twice the diameter of the egg and about one-half as deep remain isolated upwards of 24 hours; however, incubation records were taken twice each day.

Each newly hatched grub was placed in a 4-oz. tin box filled with moderately well packed rich unsterilized soil and supplied with grapenuts as food. A few finely ground granules of the latter placed in the bottom of a small cell made in the soil and the grub placed directly upon the food then immediately covered with soil was found to be the most successful procedure at this early stage of the life cycle. Fresh soil and increasing amounts of food were supplied at 3 or 4 day intervals throughout the first instar. After the first molt the grubs were transferred to larger containers, 6- to 8-oz. in size, and the interval between feeding and supplying fresh soil was increased to about 10 days. The amount of food that developing grubs require increases considerably after the second molt. During this period of maximum food requirement in the third instar carrot was used to supplement the grapenuts diet in some instances but no evidence was secured to indicate that this combination improved either growth or vigor of the individuals receiving the same. The period between observations during the final larval stage was 14 and 21 days but best results were noted when the shorter interval was used. Biweekly manipulations have no deleterious effect upon well-fed vigorous grubs. As the latter attained maturity in the fall they were removed from the rearing media of high humus content, transferred to soil taken from local fields and kept therein for the remainder of the life cycle. It was found that this procedure resulted in a marked decrease in mortality during the following spring and summer or the period during which local 2-year cycle species remain in a state of aestivation. This has proved the most critical period through which to carry the grubs successfully in close confinement and the individuals were not disturbed generally for observation at less than 30 day periods up to the prepupal stage.

When the proper combination of temperature and moisture conditions is maintained pupation and pupal development proceed with little mortality. Continuing the imposed conditions of the previous stage or using a slightly drier soil will insure the best results. The grub may be left in the larval cell or removed as a prepupa to a new one prepared in a box level full of tightly packed soil so that the lid when replaced seals the upper side of the cell. By use of the latter method it is possible to make frequent observations, seemingly without any noticeable injurious effect, upon normal pupal development. After transformation to the adult was completed the beetle was left undisturbed for 5 or 6 days; when fully hardened, as indicated by complete normal coloration, it was placed in a box of fresh soil and carried through the winter in the rearing cellar without any further attention. About 90 per cent of the beetles handled in this manner passed the winter successfully.

Phyllophaga calceata (Leconte).—The species was originally described from Texas, in the male sex only, by Leconte (1856) under the name *Lachnosterna calceata*. Horn (1887) redescribed it, includ-

ing both sexes, under the same name, repeating "Texas" as the range of distribution. Nearly 30 years later Glasgow (1916) revived the name *Phyllophaga*, proposed by Harris in 1826 for this genus, because the original publication was accompanied by a series of valid specific names. This conclusion was followed by Leng (1920) and most subsequent authors. The generally accepted valid combination for the present species is therefore *Phyllophaga calceata* (Leconte).

It is primarily a southwestern form, which occurs commonly throughout the eastern or humid region of Texas. Definite records of occurrence include about 40 counties extending from the Oklahoma to Mexican border and west to Mills and Eastland Counties. Within this large area the species is undoubtedly of considerable economic importance. Hayes & McColloch (1920) reported *calceata* as common in the southern half of Kansas and Forbes (1916) listed 10 specimens from southern and central Illinois taken only in 1907, all at lights. Langston (1927) added Mississippi to the known range of the species and listed elm, gum, hackberry, hickory, oak, pecan and willow as food plants. Post oak and elm are the preferred food plants of the adults in local areas; none have been recorded from hickory or pecan. Pin oak may be added to the list as a food plant of minor importance.

Phyllophaga calceata may be distinguished readily in both sexes from all related forms by the peculiar structure of the proximal segment of the hind tarsi. The latter segment is barely longer than wide and has the apical extremity abruptly produced on the outer side. This character is exclusive. Also, it is quite distinct without magnification so that positive field determinations of the species can be made without difficulty. The adult is a dark brown glabrous beetle, ranging from 17 to 20 mm. in length, oblong oval in form and slightly widened behind in the female. The elytra are moderately shiny, finely punctate, with sutural and first discal costae well defined except near base. Secondary sexual characters are strongly developed; males may be recognized at a glance by the broadly concave venter, which is evenly rounded in the female. The genitalia in both sexes are distinctive and have been described and illustrated by Hays & McColloch (1920), Langston (1927) & Sim (1928).

LIFE CYCLE.—Twelve field-collected beetles were placed in an oviposition cage on April 4 and supplied with fresh post oak and elm foliage daily throughout the period of their activity. The soil in the cage was screened at 2- and 4-day intervals to remove the eggs. On June 1 only one beetle remained alive and the entire lot had deposited a total of 1296 eggs or at the average rate of nearly 2 eggs per beetle day over the 56-day period. Although the beetles withstood the imposed conditions of close confinement and crowding fairly well it seems likely that under more normal conditions the number of eggs laid per female may be greater than indicated by these figures. Also, the oviposition period is undoubtedly more protracted since adult activity normally begins in February or March and late-emerging individuals may continue to oviposit well into July.

The eggs are laid singly in cells prepared by the female in small lumps of soil 2 to 8 inches beneath the surface. Newly laid eggs are pure white, elongate oval in shape and average about 2 mm. in length. They begin to swell in two or three days and gradually assume a spherical outline, reaching maximum size, 3–3.5 mm., when embryonic development has been completed. At this time the darker part of the mandibles and head capsule of the embryonic larva are visible through the thin tawny egg shell. During the hatching process the mandibles apparently rupture the shell and as the larva emerges the thin or tissue-like remainder collapses and is translucent white in color. The remains of the egg shell are not eaten by the larva.

A summary of the data recorded on the time required for incubation of 1219 eggs is presented in table 1. The duration of the egg stage varied from 26 to 43 days and averaged about 35 days over the period of observation, which extended from April 14 to June 29, 1942. During this period the average daily mean temperatures in the rearing cellar increased gradually from 62° to 68° F.

Young or newly hatched larvae average 3 to 4 mm. in length, are wholly white in color but the head soon turns brownish and after ingestion of soil and food begins the dark contents of the alimentary tract become visible through the body wall.

Table 1.—Duration of egg stage, *Phyllophaga calceata*, College Station, 1942.

Date Laid 1942	Number of Eggs Observed	Incubation Period Days			Temperature Ave. Daily Mean
		Max.	Min.	Wt. Ave.	
Apr. 14–21	12	42	41	41.3	62.6
Apr. 21–26	14	43	39	40.3	63.7
Apr. 26–29	46	42	38	39.9	64.2
Apr. 29–May 1	17	40	35	38.6	64.4
May 1–4	68	41	33	38.5	64.7
May 4–6	137	39	36	37.3	64.9
May 6–9	342	39	36	36.4	65.4
May 9–11	82	37	34	34.8	65.5
May 11–13	127	38	35	35.4	65.9
May 13–17	135	36	30	32.1	66.2
May 17–22	38	35	31	32.1	66.8
May 22–25	57	33	30	30.1	67.3
May 25–27	56	31	28	28.6	67.7
May 27–29	30	29	28	28.9	67.9
May 29–June 1	58	30	26	27.6	68.2
Average duration of incubation period				34.8	

With access to suitable food young larvae begin to feed within a day after hatching and make rapid growth. Two molts are required during the active feeding period by the grubs to attain maturity. The data recorded on larval development of the 1942 brood are briefly summarized in table 2. The duration of the first and second instars averaged approximately 47 and 38 days, respectively. The third larval instar proved much more protracted and averaged about 344 days, with extremes ranging from 305 to 368 days. In explanation it may be pointed out that this developmental stage includes not only the overwintering period, when mature grubs remain semidormant, but also a period during the subsequent warm season when they remain inactive pending the advent of conditions essential for normal pupation. Temperatures in the rearing cellar during larval development varied gradually from 53° in winter to 75° F. in summer. As determined in these studies the total larval period of *calceata* is 429 days but the feeding activities of the grubs are usually completed during about the first quarter of this period.

In preparing to pupate the larva voids the blackish contents of the alimentary tract after which the body contracts, becomes limp and slightly translucent. The prepupal stage is usually brief, ranging from 3 to 7 days. The newly transformed pupa is pallid or whitish but soon begins to assume a tawny tinge and gradually turns reddish brown in color as transformation to the adult proceeds. In size the pupa varies slightly, averaging 18 to 20 mm. in length and 9.5 to 12 mm. in width. Under natural conditions pupation occurs within the larval cell 4 to 10 inches beneath the surface of the soil. Pupation of the 1942 brood of *calceata* larvae, as observed under laboratory conditions, extended from July 25 to September 27, 1943; the bulk of the brood transformed during August. Throughout this period temperature in the rearing cellar remained practically constant at 75° F. The data secured on the duration of the pupal stage are summarized in table 3. The time required for development in this stage was found to range from 22 to 35 days and average nearly 27 days for each sex.

SUMMARY.—The life history of *Phyllophaga calceata*, as determined in these studies, may be briefly summarized as follows: Overwintering beetles emerge commonly during the latter half of March and stragglers may remain active in the field to early summer. Oviposition is well under way by mid April and extends throughout May. The incubation period averages about 35 days. The grubs feed throughout the summer, molt twice, and attain full growth by November. Mature grubs pass the winter and remain quiescent during the following spring and early summer. The average duration of the larval stage is 429 days. Pupation occurs commonly during August and about 27 days are required to complete development in this stage. The newly transformed

Table 2.—Duration of larval stage, *Phyllophaga calceata*, College Station, 1942–43.

Instar	No. of Larvae Observed	Number of Larvae in Instar for Period Specified						Weighted Average Days
		26–44 days	45–59 days	60–70 days	305–329 days	330–364 days	365–368 days	
First	266	113	146	7				46.9
Second	262	219	38	5				38.4
Third	182				22	156	4	343.8
Total larval period								429.1

Table 3.—Duration of pupal stage, *Phyllophaga calceata*, College Station, 1943.

Sex	Number of Pupae Observed	Number of Individuals in Pupal Stage for Days Specified														Weighted Average Days
		22	23	24	25	26	27	28	29	30	31	32	33	34	35	
Male	91	1	2	13	12	15	26	9	6	4	1	1		1		26.5
Female	88	1	2	3	11	32	15	12	5	1	3	1	1		1	26.8
Average duration of pupal stage																26.6

adults remain within the pupal cells during the following winter and emerge the next spring to complete a 2-year life cycle.

Phyllophaga micans (Knoch).—This form was originally described by Knoch (1801) under the name *Melolontha micans*. Blanchard (1850) and Burmeister (1855) assigned it to *Ancylonycha*. Leconte (1856) and Horn (1887) published redescriptions of the species both authors referring it to the genus *Lachnosterna*. This name remained in use until Glasgow (1916) showed *Phyllophaga* to be an older valid name. Since that time the species has been referred to in our literature as *Phyllophaga micans* (Knoch). Travis (1934) has listed the principal references.

The species is widely distributed in the United States and according to published records ranges from Massachusetts to Iowa and south to Louisiana and Georgia. It also occurs commonly in eastern Texas; especially throughout the region where the annual rainfall totals 40 inches or more. Travis and Comal Counties represent the present known western limits of the species and these records of occurrence closely approach the line dividing the humid and subhumid regions of the state. *Phyllophaga micans* is one of the early-emerging local forms and during seasons of heavy beetle flights pecan, post oak, elm and persimmon are extensively attacked and sometimes severely defoliated. Additional food plants recorded include: sweet gum, hickory, black walnut, jujube, plum, dewberry and rose. Little or nothing is known concerning the economic importance of the insect in the grub or larval stage. However, the heavy beetle emergence noted in some local areas indicates alternate season occurrence of heavy grub-populations, which may prove a factor of considerable importance in range or pasture lands and perhaps other cultivated crops.

The adult of *Phyllophaga micans* is a rufescent to dark brownish glabrous beetle 15.5 to 18 mm. in length, with a distinct pruinose sheen on elytra, pronotum and sides of abdomen. The body form in both sexes is somewhat elongate and subparallel. As usual the male venter is more distinctly flattened and the antennal club longer than in the female. Only one known species, viz., *P. sacoma*, closely approximates *P. micans* in general appearance and where these two forms range in common distinction becomes difficult except by reference to the genitalia. According to Horn (1887) Blanchard failed to distinguish *P. micans* from *P. prununculina*; however, the differences between these species are now well known and there appears little likelihood of any further confusion here. It is of interest to note that although *P. micans* has been known for nearly 150 years it has been consistently recognized throughout this period, as evidenced by the absence of any superfluous names applied to the species in literature. Horn (1887) considered *P. sororia*, described by Leconte as new in the female sex only, to be a synonym of *P. micans*; however, Glasgow (1916), who also saw the type and studied the genitalia, pronounced it the same as *P. diffinis* (Blanchard). The latter synonomy is repeated in Leng's Catalogue.

LIFE CYCLE.—Adults normally emerge early in the growing season and after a brief period of activity oviposition begins and is well under way by mid April. Field-collected adults caged in lots of 5 to 10 pairs on May 4, 1941 oviposited freely during the remainder of the month. Three pairs of reared beetles under observation in separate cages from May 5 to June 3, 1942 laid a total of 180 eggs or at an average rate of about 2 eggs per day over the period indicated. The maximum number of eggs recorded was 98; the minimum 30. Subsequent dissections of the female beetles showed the presence of additional partially developed eggs indicating that under more favorable environmental con-

Table 4.—Duration of egg stage, *Phyllophaga micans*, College Station, 1941 and 1942.

Date Laid	Number of Eggs Observed	Incubation Period Days			Temperature Ave. Daily Mean
		Max.	Min.	Wt. Ave.	
May 10–13, 1941	113	28	26	27.0	66.2
May 13–19	167	28	23	25.6	66.9
May 19–23	176	30	23	24.9	67.7
May 23–29	339	29	21	22.7	68.3
May 29–June 4	260	26	18	22.1	69.0
June 4–10	333	24	17	19.6	69.5
June 10–13	12	23	21	21.2	69.9
May 11–14, 1942	5	30	29	29.4	65.2
May 14–18	52	30	27	28.5	65.9
May 18–22	30	28	26	26.7	66.4
May 25–27	30	27	25	26.6	67.4
May 27–29	78	28	25	25.6	67.8
May 29–June 1	98	25	23	23.9	67.9
June 1–3	15	23	22	22.9	68.2
Average duration of incubation period				24.8	

ditions these individuals would have deposited a greater number of eggs than indicated above.

The manner of egg deposition in this species and the progressive changes in appearance of the egg as embryonic development proceeds are very similar to the corresponding stage described above under *Phyllophaga calceata*. The principal difference is that *P. micans* eggs are smaller when laid but they increase in size at a more rapid rate since the embryonal period is considerably shorter. The duration of the incubation period was found to range from 17 to 30 days and averaged about 25 days, as compared with 35 days in *P. calceata*. Table 4 shows a summary of the records secured on 1717 eggs laid during May and early June 1941 and 1942. The shortest incubation periods occurred when the average mean temperature ranged from 68° to 70° F.

Newly hatched larvae are small wholly white delicate grubs, averaging barely 3 mm. in length, but the head soon begins to turn brownish and after feeding activities start the body gradually becomes darker in color. During the first few days of the feeding period the grubs increase rapidly in size, provided they have access to adequate food. As growth proceeds the amount of food required increases accordingly and the grubs begin to move about more freely in the soil. In common with other related species, *Phyllophaga micans* grubs require two molts during the feeding period to attain full growth. The data secured on development of larvae of the 1941 and 1942 broods are summarized in table 5. The first and second growth periods are relatively short in duration; each averaged approximately 37 days. In the final period or third instar the grubs require an additional feeding period of about 70 days before attaining full growth. They overwinter in this stage and remain quiescent during the following spring and early summer so that the third instar, as determined in these studies, may range from 296 to 391 days. The average duration of the period, based upon 123 complete rearing records, was 339 days. During the period of larval development temperatures varied gradually with the season from 53° in winter to 75° F. in summer. Although these data indicate that *P. micans* normally requires 2 years for the development of a complete generation it may be of interest to point out that in several instances larvae were noted to pupate and transform to adults shortly after attaining full growth, indicating a 1-year cycle for these specimens. However it appears unlikely that this abbreviated life cycle occurs any more commonly under field than laboratory conditions and therefore is of little or no practical importance.

The process of transformation from larva to pupa in *Phyllophaga micans* is similar to the corresponding preparatory stages described under *calceata*. However, *P. micans* pupae are less robust in form, averaging 16 to 18 mm. in length and 8 to 10

Table 5.—Duration of larval stage, *Phyllophaga micans*, College Station, 1941–42 and 1942–43.

Instar	No. of Larvae Observed	Number of Larvae in Instar for Period Specified						Weighted Average Days
		22–44 days	45–59 days	60–79 days	296–329 days	330–364 days	365–391 days	
First	430	405	25					37.2
Second	429	347	47	35				36.9
Third	123				31	80	12	339.0
Total larval period								413.1

Table 6.—Duration of pupal stage, *Phyllophaga micans*, College Station, 1942 and 1943.

Sex	Number of Pupae Observed	Number of Individuals in Pupal Stage for Days Specified												Weighted Average Days
		19	20	21	22	23	24	25	26	27	28	29	34	
Male	63	2	4	9	18	13	9	3	1	3			1	22.5
Female	60		7	7	18	6	5	7	3	3	2	2		22.9
Average duration of pupal stage														22.7

mm. in width, and require considerably less time for complete development. Larvae of the 1941 brood pupated from July 18 to September 12, 1942 and those of the 1942 brood from July 25 to August 30, 1943. During both seasons the majority of individuals transformed during August. During these periods of observation temperatures in the rearing cellar averaged from 73° to 75° F. A summary of the data recorded on the duration of the pupal stage is presented in table 6. Under conditions of close confinement the time required for pupal development was found to range from 19 to 34 days and averaged approximately 23 days for both sexes.

SUMMARY.—The history of a complete generation of the present species under local conditions is about as follows: Overwintering beetles emerge in maximum numbers during April and eggs are commonly present during the latter part of this month and throughout May. About 25 days are required for the eggs to hatch. The larvae continue to feed through the summer and after molting twice attain full growth by September or October. Normally the mature grubs pass the winter and remain quiescent during the following spring and summer until after mid July, when pupation begins. The average duration of the larval stage is 413 days. Pupation begins during the latter half of July and pupae are commonly present during August and September. About 23 days are required in this stage for transformation to adults. The latter remain within the pupal cells throughout the following winter and emerge the next spring to complete a 2-year life cycle.—4-13-44.

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The Relation Between Host Condition and Attacks by the Bronzed Birch Borer

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The entomological literature of North America contains numerous accounts of the destruction of ornamental birches by the bronzed birch borer, *Agrilus anxius* Gory. Some of these accounts contain original notes on the life history, *i.e.*, Chittenden (1898), Larsen (1902), Slingerland (1906), Swaine (1917) and Hutchings (1922–23), but none contain experimental proof that the damage was caused primarily by the borer. Their conclusions were based on observations of infested dying trees. On the other hand, those who have studied this insect in forest trees have not always found it to be the primary cause of the death of the trees. In two of these studies, Spaulding & MacAloney (1931) and Hall (1933) concluded that the insect was attacking because the trees were dying. Swaine (1918) and Balch & Prebble (1940), however, have reported this insect to be attacking and killing many healthy mature birches in Canadian forests.

When trees are already infested it is often difficult to determine whether the insects have caused the decadence or whether they are present because the trees are in a poor condition. For the present study, young, vigorous, uninfested trees were selected, and then injured in various ways so as to produce decadent conditions. The attractiveness of these injured trees for the beetles and their suitability for the development of the larvae were observed and compared with the same characteristics for uninjured trees. This experimental method was suggested by F. C. Craighead after it had been observed that many aspen snags, caused by a windstorm the previous summer, were infested with larvae of the bronzed birch borer.

The work was done in northern Minnesota, where more than half of all forest land is occupied by the aspen-birch type (Kittredge & Gevorkiantz 1929). In this type, quaking aspen, *Populus tremuloides* Michaux, is the dominating species, while paper birch, *Betula papyrifera* Marshall, is the most abundant associate. *Agrilus anxius* breeds in both these hosts, in addition to other species of these genera.

During the third week of June 1941, 18 dominant and codominant aspen trees were topped at a height of 11 feet, and 12 others were girdled close to the ground. The trees listed as felled were the tops removed from the topped trees. One-third of the topped trees were also girdled near the base of the trunks. Twenty-five birch trees (15 topped and 10 girdled) were treated in the same way. Undamaged trees of both species were used for checks. In the mixed aspen-birch stand many of the birches were in poor condition and contained welts indicating old attacks by the bronzed birch borer. Because of this, vigorous uninfested birch trees were selected from a small stand of pure birch about 1 mile from the aspen. Both the aspen and birch stands were about 31 years old, well stocked, and on sites of medium quality. The trees were between 4 and 7 inches d.b.h.

Standard statistical procedures were used to determine whether the differences between the various treatments were significant. Analysis of variance techniques were especially useful.

ATTRACTION OF BEETLES TO DIFFERENT TYPES OF HOST MATERIAL.—Beginning a few days after treatment, the trees were observed daily for several days to find out whether they were more attractive to the beetles than were undamaged trees. Each observation consisted of counting the number of beetles present on the trees during the time of observation (approximately 30 seconds). These data were all collected on sunny days between 10 a.m. and 3 p.m., with the shade temperature between 75° and 90° F. Many of the beetles observed were in copula and a few females were ovipositing. The data in column *A* of table 1 show that the beetles were more strongly attracted to the damaged trees.

From the data presented in table 1 it

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Table 1.—Attractiveness, population density, and gallery characteristics for *Agrilus anxius* in different types of aspen host material.

Condition of Host Trees	Average Beetles per Tree per Observation[1]	Observations	Living Larvae per Square Foot	Prepupae, Pupae, and Adults per Square Foot	Trees Observed	Average Gallery Length	Average Departures Made into Xylem	Observations
	Number A	*Number*	*Number* B	*Number* C	*Number*	*Inches* D	*Number* E	*Number*
1. Vigorous	0	67	0	0	5	—	—	—
2. Suppressed	0	21	0.1	0	10	51.2	5.2	15
3. Girdled	0.1	54	.7	0	12	36.4	4.1	13
4. Topped	1.9	54	.6	1.2	12	24.4	3.0	37
5. Topped and girdled	2.8	27	.2	2.7	6	23.8	2.5	4
6. Felled (lopped)	1.0	27	0	1.7	6	22.6	2.2	20
7. Felled (not lopped)	1.3	27	0	1.1	6	19.5	0	27

[1] Values corrected so that each represents the number of beetles observed per 17 square feet of bark area. This was the average area per tree.

The differences between any of the following are not significant: Column *A*—1 and 2, 4 and 5, 6, and 7; the sum of columns *B* and *C*—any combination of 3, 4, 5, 6, and 7; column *D*—any combination of 4, 5, and 6, and any combination of 5, 6, and 7; column *E*—4 and 5, and 5 and 6. In all other cases the differences are highly significant.

was concluded that the suppressed trees were unattractive to the beetles; however, in another experiment the observation period was extended to 14 days, therefore a better test was obtained. This was accomplished by attaching sheets of flypaper around the trunks of 10 pairs of aspen trees. Each pair consisted of adjacent trees—one vigorous and one suppressed. In no pair were the trees separated by more than 8 feet, and in 6 of the pairs the trees were not over 4 feet apart. During the 14-day period the 5 square feet of flypaper on the suppressed trees caught 18 bronzed birch borer beetles, while no beetles were caught by the flypaper attached to the vigorous trees. This result shows that the beetles were more strongly attracted to the poorer of the undamaged trees.

The birch trees were observed at different times on several days and no beetles were seen. This, together with the small larval and pupal population (Table 2), indicates that the beetle population was smaller in the stand of birch than in that of the aspen.

To determine whether the population difference in the stands of aspen and birch might be due to a racial difference of the beetles, characterized by each race limiting its attacks to only one of the host genera, an experiment was set up in the aspen-birch stand. Six pairs of similar-sized, vigorous aspen and birch trees were felled and lopped early in June, the trees of each pair being from 10 to 20 feet apart. In August the bark was stripped from a portion of each of these trees (44 square feet, or 20 per cent, from the aspen and 29 square feet, or 13 per cent, from the birch), and the larvae were counted. There were 1.6 larvae per square foot for the aspen and 3.3 per square foot for the birch.

Table 2.—Population and gallery characteristics of *Agrilus anxius* in various types of birch host material.

Condition of Host Trees	Living Larvae per Square Foot	Prepupae and Adults per Square Foot	Trees Observed	Average Gallery Length	Average Departures Made into Xylem	Observations
	Number A	*Number* B	*Number*	*Inches* C	*Number* D	*Number*
1. Vigorous	0	0	5	—	—	—
2. Girdled	0.24	0	10	33.3	4.0	6
3. Topped[1]	.02	0.03	10	—	—	—
4. Felled (lopped)	.01	.61	5	16.3	0	16
5. Felled (not lopped)	0	.02	5	—	—	—

[1] The "topped and girdled" birches were uninfested.

In columns *A* and *B* the differences for the total population between any combination of 2, 3, and 5 are not significant; in all other cases the differences are significant. The difference in the gallery lengths is significant.

The difference was not significant.

In this aspen-birch stand practically all the beetles present had emerged from standing aspen snags caused by a windstorm in the latter part of July 1940. They were lightly infested the first season and became heavily infested the following year. About 4 per cent of the basal area of the aspen was broken, while the tougher birch was undamaged. From these snags large numbers of beetles emerged in June 1942. Counts made on 10 snags (131 square feet of bark surface) revealed that the average number of beetles produced was 3.0 per square foot of bark area. Thus, in an area where the beetles developed in aspen the felled trees were not more heavily attacked than the felled birch. This indicates that there was no preference of the beetles reared in trees of one genus to limit their attacks to that host; therefore, we were not dealing with two biological races of the bronzed birch borer.

Adults of the bronzed birch borer are often seen on trees exposed to full sunlight. From counts made of the number of beetles on the topped and felled trees at times when they were exposed to various light conditions, the following results were obtained: 2.6 beetles per tree per observation (26 observations) when the trees were exposed to full sunlight, 1.8 beetles (86 observations) when the trunks were partially shaded, and 1.0 beetles (42 observations) when the trunks were completely shaded. The differences were significant or highly significant. The following season, however, results of 180 similar observations made on felled aspen and birch did not support the data given above. During the first season topped trees were abundant and the beetles were able to make a selection, whereas in the second year decadent host material was scarcer. This suggests that host suitability is more important than the amount of sunlight on the trees.

POPULATION DENSITIES IN DIFFERENT TYPES OF HOST MATERIAL.—During the latter part of September 1941, the same year in which the trees had been damaged, a portion of the bark (22 per cent of the total area sampled) was removed and the larvae and prepupae were counted. The following spring, samples of the trees (12 per cent of the total area sampled) were placed in cages and the emerging adults caught. Snags and felled trees containing the remaining 66 per cent of the bark area were left in the woods, where the adults were allowed to emerge. After emergence was completed the exit holes were counted, the bark was removed, and the living larvae were counted. The total area sampled averaged about 10 square feet per tree. Table 1 (columns *B* and *C*) and table 2 (columns *A* and *B*) contain the combined data collected in these three different ways.

The "topped and girdled" aspen contained the largest population but, as the mortality was 100 per cent in one of the six trees, the living population at the time of sampling was not significantly different from that of the other damaged trees. No prepupae, pupae, or adults were found in the girdled trees (on the trunk above the girdle) because it took 2 years for the larvae to complete their development in these trees, and at the time of the examination only 1 year had elapsed. This effect of the condition of the host on the life cycle will be discussed more fully later.

One overgrown gallery was found in each of four of the five vigorous check aspen trees. These galleries were covered by the growth of seven annual rings; therefore, the attacks occurred in 1934, the sixth consecutive year of deficient rainfall, which ranged from 5 to 34 per cent below average for the 6 years. During 5 of the years the deficiency was 11 per cent or more.

In the suppressed aspen trees, in addition to the living larval population (Table 1), there were 0.2 gallery per square foot of stem area examined in which the larvae had died and the galleries were overgrown by subsequent woody growth. Healed-over galleries are shown in figure 1.

The larval and pupal populations in the different types of birch host materials are shown in table 2. The felled trees with the branches lopped were the most heavily infested.

By the seventh of May more than half of the prepupae had pupated and by May 19 all were pupae. Beetle emergence from the caged aspen and birch logs began on May 29 and ended on July 2, the peak occurring on June 10. From these caged logs, 345 beetles were obtained, 56 per cent of which were females.

Woodpeckers removed about 10 per cent of the prepupae from the infested trees left in the woods over winter. The extent of parasitization of the bronzed birch

FIG. 1.—Healed-over galleries in paper birch. Seven annual rings cover the galleries. (Slightly reduced.)

FIG. 2.—Complete gallery in a topped aspen. Arrow 1 points to the beginning of the gallery at the cambium-xylem interface. The visible sections of the gallery are joined by invisible portions present below the surface of the wood. (Slightly reduced.)

borer was not accurately determined because the logs used for rearing also produced a few curculionids and xiphydriids; however, six adults of a parasite, *Phasgonophora sulcata* Westw., issued from cocoons removed from pupal cells of *Agrilus anxius*.

The average length of the beetles reared from aspen was greater than that of those reared from birch. Fifty-five males reared from aspen averaged 8.7 mm., whereas the 8 reared from birch averaged 8.2 mm. The 48 females reared from aspen averaged 9.5 mm. and 7 females reared from birch averaged only 8.8 mm. The difference in length was significant for the females, but not for the males. No other differences were discernible in the specimens reared from the two hosts. The identification of specimens reared from both hosts was verified by W. S. Fisher, of the Bureau of Entomology and Plant Quarantine.

DIFFERENCES IN LARVAL GALLERIES IN VARIOUS TYPES OF HOST MATERIAL.—The oviposition sites were usually the roughest portions of the stem and were frequently associated with branch scars. The females deposited their eggs in the bottom of cracks in the bark. When the bark was removed from these places the origin of the galleries could be seen easily. From 14 observations it was found that

the number of larvae starting from one place ranged from 1 to 5, averaging 2.4. The larvae penetrated the bark and cortex directly. After they reached the phloem they spent a variable length of time in it. Some passed through this tissue almost directly to the cambium-xylem interface, whereas others traveled obliquely as much as 10 inches before they reached the cambium and began to etch the wood. The average distance traveled was 5.5 inches.

After a larva reached the cambium it bored there during most of the remainder of its larval life; however, in some types of host material the larvae, at periodic intervals, left the cambium and burrowed through the wood for short distances (Fig. 2). After a short stay in the wood, they again returned to the cambium. The number of times the larvae did this depended on the condition of the host material. The more severely the trees were damaged, the fewer the number of departures into the xylem (Tables 1 and 2).

Chapman (1915) first pointed out for another species of the same genus, *Agrilus bilineatus* Weber, that these departures into the wood were made for the purpose of ecdysis. Hall (1933) believed that this is also true for *A. anxius*, while Balch & Prebble (1940) questioned this interpretation.

In the present study it was noted that these major departures into the xylem occurred at regular intervals. During the first period in which a larva is at the cambium-xylem interface it constructs a very short gallery segment. With each successive period the gallery segments become longer (Fig. 2). In the 15 galleries measured, the average lengths of these gallery segments in topped aspen for the successive periods of larval growth were as follows: 1.3, 3.9, 7.1, and 13.0 inches. This was also true of similar data for galleries in suppressed trees. These results show that there is a definite rhythm related to the time when the larvae leave the cambium and bore into the wood. In addition, a series of width measurements were made at regular intervals along 12 galleries in aspen in which the larvae had bored into the wood at three different times. From these data it was found that the average rate (in mm. per cm. of gallery length) at which the width of the galleries increased when the larvae were in the cambium was 0.03, whereas the rate of increase when the larvae were boring in the xylem was 0.16. The difference was highly significant. In a gallery in a suppressed tree, the portions which dipped into the xylem were carefully cut apart and the boring dust was carefully examined. Parts of exuviae were found in all but one of the departures into the xylem. These results led the writer to believe that molting was the primary reason for the major detours into the wood; however, when the host trees are in a certain condition the larvae do not exhibit this characteristic (Table 1, column *E;* Table 2, column *D;* and Fig. 3).

The larvae bored into the wood about 0.25 inch in the topped trees and 0.6 inch or deeper in the suppressed trees. In some instances where the larval population was very dense the larvae went into the wood an additional number of times when approaching full growth. These departures, however, were characterized by being very shallow and very short.

In birch the larvae usually formed winding galleries, whereas both winding and zigzag galleries were formed in aspen. The latter type was often very compact in trees that were not damaged too severely; *i.e.*, those that were suppressed, girdled, or topped (Fig. 2), but was much less frequent in the felled trees.

When the population was dense, the larvae, as they grew older, usually lost the habit of compacting their galleries (Fig. 3). The more rapid disintegration of the inner bark under these conditions may have been the reason for the change.

It was also noted that there were differences in the larval galleries in the different types of host materials. The galleries became shorter as the severity of the treatment increased, as shown by the data in column *D* of table 1, and column *C* of table 2. In making the measurements, it was necessary to select galleries that were somewhat isolated, in order to follow their course. When the inner-bark tissues had already turned brown before the galleries were measured, their course through the phloem could not be followed. For this reason the gallery lengths given do not include the portion formed when the larvae passed through the phloem.

In growing trees the complete development of larvae was usually unsuccessful. Only two galleries in the living suppressed trees were complete, and in these the pre-

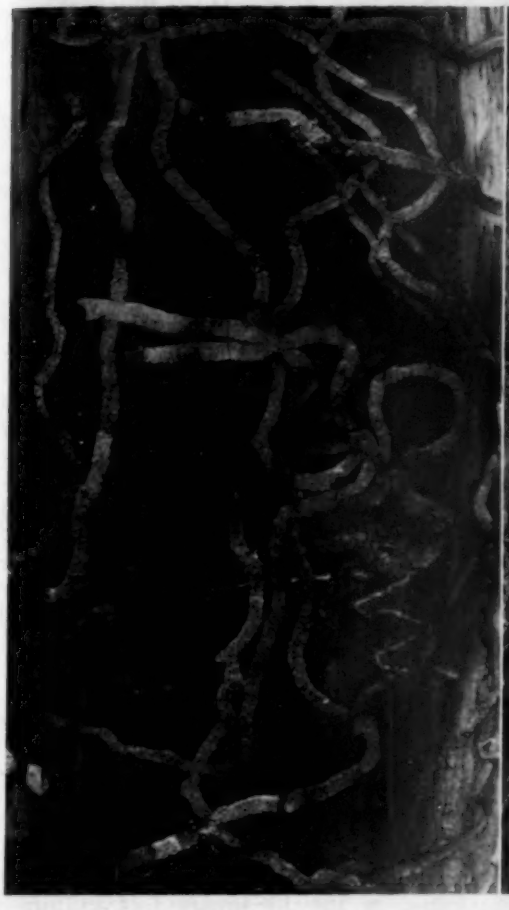

FIG. 3.—Galleries formed by older larvae in a heavily infested topped aspen. Prepupal density was 8.5 per square foot. (Slightly reduced.)

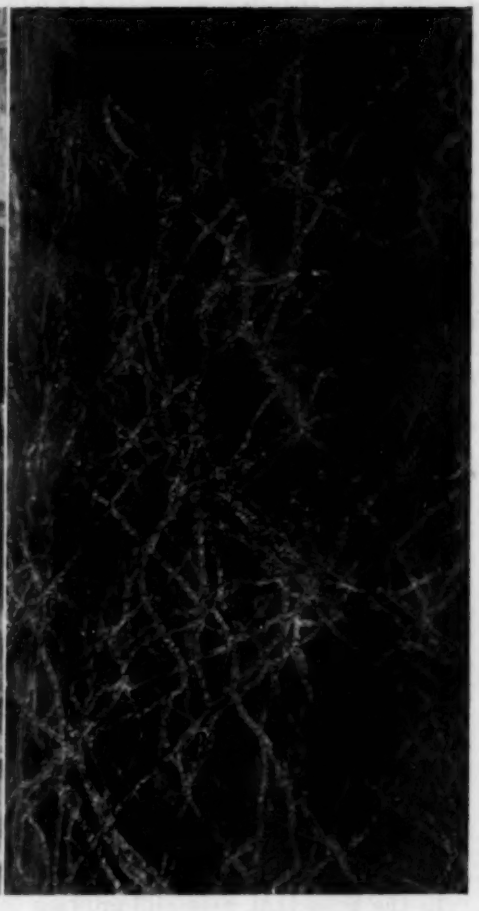

FIG. 4.—Concentration of larval galleries above the girdle in a topped and girdled aspen. Larval mortality was 100 per cent in this portion of the tree, probably because the inner-bark tissues had disintegrated too rapidly. (Slightly reduced.)

pupae had died. Because completed galleries were so scarce in the suppressed and girdled trees, incomplete galleries containing older larvae were measured.

RELATION BETWEEN CONDITION OF HOST AND LENGTH OF LIFE CYCLE.—Slingerland's (1906) observations led him to believe that the bronzed birch borer had a 1-year life cycle. Swaine (1917) noted that at least some individuals had a 2-year life cycle. This was substantiated by Spaulding & MacAloney (1931), who found that most of the individuals in the trees they were studying took 2 years to attain maturity. Hall (1933) and Balch & Prebble (1940) also reported a 2-year life cycle.

In the present investigation it was found that the length of the life cycle was either 1 or 2 years. In the topped-and-felled trees a 1-year life cycle predominated. Of the total living population present when the topped and felled aspen and birch were examined, 83 per cent completed their development in 1 year. Had these trees been initially infested in 1940, the early 1941 growth (1.0 mm.) would have started to heal over the galleries produced in 1940. There was no evidence of this on any of the galleries in these trees. It was frequently observed, however, in the suppressed trees, where the average radial increment averaged only 0.2 mm. for the entire year. These observations, together with the data already given on population density in the various

trees, show that in topped-and-felled trees the life cycle is usually 1 year. This includes the treatments 4, 5, 6, and 7 in table 1, and 3, 4, and 5 in table 2.

In these topped trees the living larvae that had not completed their growth by the end of the first summer were about half grown; therefore, they probably had hatched too late in the season to complete their development before cold weather. This suggested explanation is supported by an observation on some aspen trees broken in the latter part of July 1940. The broken trees were lightly infested the same summer they were damaged, yet no adults emerged until 1942. This indicates that when the attacks occur too late in the summer, even in the more suitable breeding material, the larvae have to spend two winters in the trees; however, when the eggs are laid in June or early in July on nongrowing trees, the life cycle is completed in 1 year in both the quaking aspen and the paper birch.

Only prepupae, pupae, and adults were found in the felled aspen and in the felled birch that were not lopped. No living larvae were found in these trees, probably because the inner-bark tissues had died and changed to dark brown. When this occurred, the partially grown larvae died, but those that had finished feeding were not affected. This relationship will be discussed more fully later.

In the trees that were still growing in diameter, very few larvae lived to pupate, and the ones that did required at least 2 years to complete their development. All the older larvae found in the suppressed aspen trees had been there for 2 years. When the bark over these galleries was removed, the current year's burrows were at the cambium-xylem interface, while the first year's burrows were overgrown with woody tissues. The girdled aspen and birch trees were infested the same year they were damaged (1941), yet no beetles emerged from these trees the following summer. Probably the suppressed and girdled trees were not infested early enough in the season to enable the larvae to complete their development before winter, or it may have been that the proliferating cambial tissues had a deterrent effect. In general, however, it seemed that a cessation of the growth of the host tree during the active season was necessary for the successful completion of the cycle.

THE EFFECT OF HOST TISSUE PROLIFERATION AND LIFE OF CAMBIAL CELLS ON SUITABILITY FOR LARVAL DEVELOPMENT.—In the middle of September 1940, six aspen trees and six birches were girdled. Half the trees were girdled at d.b.h., the remainder at about 12 feet. The lowest leaf-bearing branches were at a height of about 25 feet. All the trees produced leaves the following year, but did not leaf out in the spring of 1942.

When these trees were examined in May 1942, it was found that in the girdled aspen the larval population below the girdle averaged 1.2 per square foot in an examination of 52 square feet, whereas above the girdles the average was 0.1 per square foot for the 88 square feet examined. About half the larvae had died before the trees were examined (46 per cent of the population below the girdles and 57 per cent of that above). Eighty-six per cent of the galleries found above the girdles were in one tree, which had died soon after leafing out the summer after it was girdled. Below the girdles all the living insects were either pupae or adults, while those working above the girdles were still larvae. Similar results were also obtained for the birch. In the two trees that were infested the larval population had been 1.3 per square foot for the 10 square feet examined below the girdle, while on the same trees no larvae were found in the 13 square feet examined above the girdles. Larval mortality was 65 per cent. All living larvae had pupated.

Each of the girdled trees produced a small 1941 annual ring above the girdle but none below. This growth ring, or that for any other year, could be located easily by making use of a pair of extremely small annual rings for 1937 and 1938. These small rings for the 2 years were caused by a complete defoliation of the stand by the forest tent caterpillar, *Malacosoma distria* Hbn. These trees had been girdled so late in the season of 1940 that it was impossible for them to have been infested that year; therefore, the complete larval development took place in 1941, when the cambial cells below the girdles did not proliferate.

These results show that the attacks were heavier on the portions of the trees in which the cambial tissues had stopped proliferating and that in tissues in this condition the larvae could successfully

complete their development. The opposite was true for the trees that were still growing in diameter. This conclusion is substantiated by the results obtained regarding the attacks and development of the larvae in the topped and suppressed trees (Tables 1 and 2). Even in suppressed trees where cambial tissues were proliferating very slowly (average width of annual ring, 0.2 mm.), the conditions were unfavorable for complete larval development.

The fundamental reason why proliferating tissues, or the associated conditions, are unfavorable for the development of the larvae was not thoroughly studied. It seemed, however, that moisture content was not the determining factor. In one of the aspen trees that was girdled at 12 feet, the moisture content of the inner bark was 238 per cent (dry-weight basis) below the girdle and 167 per cent above. There were 3.2 prepupae per square foot on the trunk below the girdle but no larvae or galleries were found on the portion above. The average moisture content of the inner bark of two undamaged trees was 194 per cent. In some gas-analysis studies, which were being conducted on infested topped trees, it frequently happened that the gas-collecting bottles filled with sap instead of with the stem gases. It was also noted several times that when a knife was inserted into the standing infested snags the sap would squirt out and run down the bark. On the other hand, the felled trees that were not lopped continued to lose water by transpiration until the leaves dried, causing the inner-bark tissues to become much drier than similar tissues in the undamaged trees. The success of the attacks in both the moister and the drier materials (with the exception of the felled but not lopped birch) suggests that the actual moisture content can deviate considerably before it limits the development of the larvae of the bronzed birch borer.

A number of other physiological changes probably occur in these trees when the cambial tissues stop proliferating. If these were measured and studied in relation to the success of this borer, the limiting factor or factors could probably be determined.

Even though the data presented up to this point show that the most suitable trees for the development of the bronzed birch borer were those not growing, it was found that the cambial tissues had to be alive if the larvae were to complete their development successfully. Balch & Prebble (1940) observed that the young larvae are " . . . dependent for nutrition during the greater part of their existence on the cambium and young wood and phloem on either side of it, in stems or branches which are living, or at least are still 'sappy' and undiscoloured." In the present study it was also observed that inner-bark tissues that had turned brown were unsuitable for larval development. Almost all the larvae (96 per cent) found at the cambium-xylem interface (excluding those already in pupal cells) in areas of brown inner-bark tissues were dead, while only 1 per cent were dead in the areas that were still white. The data were collected in the autumn after most of the living larvae had finished feeding and had formed pupal cells. The fully grown larvae in the pupal cells were not killed when this color change occurred; however, it was observed that in trees where the sapwood had started to rot the mortality was heavy, even for those in pupal cells. An outstanding example of heavy mortality due to the browning or disintegration of the inner-bark tissues occurred in one of the "topped and girdled" aspen trees. Above the girdle the inner-bark tissues were dark brown 3 months after the tree had been damaged. The population in this part of the tree had been exceedingly heavy (Fig. 4) but no living larvae could be found when the tree was examined. Below the girdle the phloem and cambium were white and contained 14.9 living full-grown larvae and prepupae per square foot. This was the densest population observed in any tree.

The change in color of the inner-bark tissues was found to be associated with the death of the cells in the cambium. This was determined by staining microscopic sections of these tissues with a vital dye. The technique was essentially the same as that used by Lorenz (1939). The staining procedure was very simple. It consisted of immersing the sections for several hours in a 0.2 molar solution of calcium chloride containing 5 p.p.m. of neutral red. Only living cells are stained by this dye. Using this technique, the writer found that the cells in the cambium remained alive as long as the inner bark remained light in color. By the time the tissues turned brown all the cambial cells were dead.

The cambial tissues remain alive for

some time after a tree is felled. In one instance the cambium was still alive 11 months after the tree had been cut. Usually, however, these tissues die sooner in felled trees. After 3 months the inner bark of the felled (not lopped) trees was in a more advanced stage of death and disintegration than in those that were felled and lopped. In the former the browned area was continuous over the whole bole, while in the latter white patches still remained. In all the other damaged trees, except one topped and girdled tree, the inner-bark tissues were still alive.

Even though the cambial tissues died sooner in the felled trees, they remained suitable long enough, except in most of the felled birch that were not lopped, for most of the larvae to complete their development. In some of the aspen and most of the infested birch the attacks were so light that the fate of each larva could be determined. The larval mortality in these trees was as follows: In four felled aspen, 26 per cent; in four topped aspen, 57 per cent; in three felled and lopped birch, 25 per cent; in three topped birch, 30 per cent; and in three felled (not lopped) birch, 86 per cent. All the trees were in well-stocked stands, therefore they were not exposed to full sunlight for any extended period of the day. Observations on the felled aspen and birch showed that the rate of dying of the inner-bark tissues was faster in the birch than in the aspen. This suggests a reason for the heavy mortality in the felled (not lopped) birch. In this material the larvae died when they were about two-thirds grown. The inner bark in these trees died too soon to permit the larvae to complete their development. This tissue disintegration suggests a reason why the gallery characteristics are similar for the felled (not lopped) aspen and the felled and lopped birch. (Tables 1 and 2.)

SUMMARY AND CONCLUSIONS.—The bronzed birch borer, *Agrilus anxius*, is a subcortical-feeding insect which is not very aggressive and which can successfully develop only in extremely decadent trees. Data are presented which show that the adults were attracted to and attacked the decadent (suppressed, girdled, or topped-and-felled) quaking aspen and paper birch trees. The most decadent trees were the most suitable for attack. They were the trees that had stopped producing radial increment (felled or topped trees and portions of the stem below the girdle on girdled trees). The decadent trees that were still growing in diameter (suppressed trees and portions of the stem above the girdle on recently girdled trees) were only lightly attacked. Evidence is submitted which shows that even the poorest of the growing trees were unsuitable for the development of the larvae. On the other hand, trees that had been dead so long that the phloem tissues were dead (brown) were also unsuitable for larval development. The results of this study suggest that whenever *A. anxius* is found in dying trees it should not be considered the primary cause of the death of the trees until all other factors that may be responsible have been evaluated.—6-10-44.

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Effect of Soils, Cover Crops, and Foliage on Concentration of HCN in Citrus Fumigation

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In field studies on the control of the California red scale, *Aonidiella aurantii* (Mask.), with hydrocyanic acid gas, conducted at the Whittier, Calif., laboratory, sampling of the gas-air mixture under the fumigation tent indicated a wide variation in the concentration from tree to tree. A study of the behavior of the gas under a fumigation tent (Fulton *et al.* 1941) revealed that when the hydrocyanic acid was released by the commercial methods there were regions of high and low concentration, and that spatial nonuniformity persisted throughout the exposure. It was shown further that, although the distribution of the gas under the tent could be improved by using a blower applicator, samples taken 30 seconds after the release accounted for only 60 to 70 per cent of the hydrocyanic acid introduced. This large loss suggested an initial sorption of the gas by the cover crop, soil, and tree foliage, in addition to loss through the tent fabric. An experiment was therefore undertaken to determine the effect of such surfaces on concentrations of hydrocyanic acid gas during a fumigation.

A 6-foot cubical covering on an edge was built of plywood to provide a portable gastight unit with a constant volume, (Fig. 1). The seams and joints were sealed with a flexible plastic compound, and the interior was treated with a hard-drying varnish that has been found to sorb a minimum of hydrocyanic acid. The box was provided with four sampling outlets on the vertical center line of each of three sides, located respectively at the bottom and 2, 4, and 6 feet above the bottom. A removable gastight bottom was provided for use in some of the tests.

A small blower atomizer was constructed for using liquid hydrocyanic acid. The liquid was measured in a calibrated chamber, and forced with an air pump through a small spray nozzle mounted in the air stream of the blower. This unit was attached to the outside of the box and could be replaced by a gastight cover. In all the tests except those in which potted citrus plants were present, the dosage was within the range of commercial usage. The fumigations were of 20 minutes duration, and 1-liter samples were aspirated 0.5, 3, 6, and 20 minutes after release of the gas. Circulation with the blower was continued throughout the fumigations.

The sorption by the walls of the box

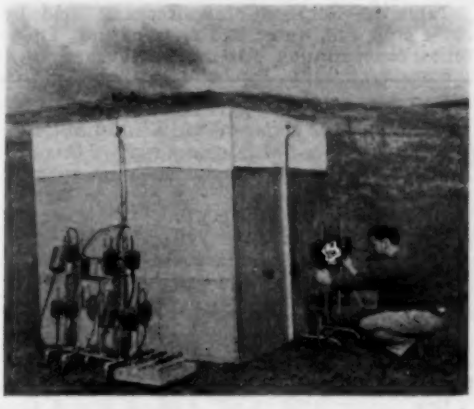

FIG. 1.—Gastight fumigation box, showing location of outlets and sampling units.

was determined with the plywood bottom in place by making a number of consecutive releases on the same day, with thorough airing between fumigations. Several tests were also made in which, after sampling for the usual period, sufficient additional hydrocyanic acid gas was introduced to restore the original concentration. This was done to see whether, after the initial sorption of gas by the walls, the concentration would fall off more slowly. However, no difference was detected in the rate of decrease.

To determine the effect of sorption by soil, the box was placed on the bare soil and sealed at the bottom by packing moist dirt firmly around the edges. Tests were made with both dry and wet soil. In a similar manner, the effect of a cover crop was studied by placing the box over a dense stand of white-stem filaree, *Erodium moschatam* L'Her., about 24 inches high. This was done both when the plants were very wet with dew and when they were

dry. In another test, to determine the effect of a large amount of moisture, water was sprayed over the inside surfaces of the box until approximately 6 inches of water accumulated in the bottom. The results of these fumigations are shown in table 1. Except where noted in the table, the concentration for a given time represents the average of four gas samples from different locations in the box, approximately 12 inches from the side. In the test with the cover crop, four samples were taken in the atmosphere above the foliage and two within the foliage.

Table 1.—Sorption of hydrocyanic acid by cover crop, soil, water, and plywood box. Theoretical concentration, 4.10 mg. per liter.

Material in Bottom	Concentration of Hydrocyanic Acid (Milligrams per Liter) After—				Average Concentration $\Sigma_0^{20} C\Delta t$
	minutes				
	0.5	3	6	20	20
White-stem filaree:					
Dry	3.56	3.72	3.40	2.49	3.14
Wet	3.46	3.42	3.32	2.51	3.06
Dry[1]	3.29	3.31	2.97	2.46	2.87
Wet[1]	3.14	3.12	2.92	2.57	2.84
Soil:					
Dry	3.90	3.89	3.66	2.82	3.41
Wet	3.83	3.75	3.49	2.79	3.31
Water	3.92	3.82	3.68	2.86	3.43
Plywood	3.73	3.67	3.57	2.99	3.39

[1] Samples were taken only within the foliage approximately 3 inches from the ground and 18 inches from side of box. Concentration represents the average of two samples.

Practically no difference was observed between the concentrations obtained with the plywood bottom in place and with the box over dry or wet soil or water. There was little difference in sorption by the wet and dry plants. The gas concentration within the foliage of the cover crop was below the average of the box for the first 6 minutes, but by the end of 20 minutes it had reached equilibrium with the atmosphere above the foliage. The presence of the cover crop was accompanied by only slightly lower concentrations than were obtained with the plywood bottom in place.

In another series of fumigations two potted orange trees were placed inside the box on the plywood bottom. To prevent severe burning of the trees the dosage of hydrocyanic acid was reduced to half that used previously. The sorption by the trees was compared with that effected by two large strips of burlap suspended from the top of the box and by the box alone. The burlap was used both wet and dry. The results are shown in table 2.

Table 2.—Sorption of hydrocyanic acid by orange trees and by burlap. Theoretical concentration, 2.05 mg. per liter.

Material in Box	Concentration of Hydrocyanic Acid (Milligrams per Liter) After—				Average Concentration, $\Sigma_0^{20} C\Delta t$
	minutes				
	0.5	3	6	20	20
Two small orange trees	1.77	1.73	1.67	1.34	1.57
Dry burlap	1.73	1.69	1.54	1.28	1.49
Wet burlap	1.75	1.56	1.44	1.12	1.37
None	1.71	1.67	1.59	1.32	1.52

There was no appreciable difference in the concentration obtained with the box alone and with the two orange trees or the dry burlap. Wet burlap reduced the concentration a little.

SUMMARY.—A study has been made of the effect of sorption by soils, cover crops, and citrus-tree foliage on the concentration of hydrocyanic acid gas during a fumigation. The loss due to sorption by such surfaces is negligible compared with the probable initial loss through the tent fabric at the time the fumigant is released. —5-5-44.

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Wheat Flour as an Adhesive for Sprays

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Wheat flour has been regarded as possessing some merit as a spreader-sticker for insecticidal sprays for many years. Since little information of a critical nature concerning its use for this purpose is available, an investigation of the problem was undertaken. This report is concerned principally with laboratory tests of the influence of wheat flour, both alone and in combination with certain other materials, upon the tenacity[2] of spray materials applied in the form of suspensions.

MATERIALS AND METHODS.—The methods employed were an adaptation of techniques used by various workers (1, 2, 3, 4, 5, 6, 7, 8) in laboratory studies of supplementary spray materials. Briefly described, a suspension containing the adhesive was atomized onto a surface, and tenacity increase due to the adhesive determined. Insoluble copper sulfate was included in the spray as an indicator of the quantity of spray residue retained on the surface since its presence could be determined readily and accurately in small quantities by chemical analysis.

The insoluble copper sulfate[3] contained 53 per cent copper; the wheat flour was an unbleached second clear containing 15.7 per cent protein; the soybean flour contained 49.8 per cent protein; and the lime 27.6 per cent calcium hydroxide. Based on the hypothetical chemical combination of the acid and basic ions, the approximate composition of the tap water was as follows (data expressed in parts per million):

Magnesium sulfate	8.
Calcium sulfate	114.
Calcium bicarbonate	190.
Calcium carbonate	117.
Sodium bicarbonate	67.
Sodium chloride	28.

SURFACES.—The artificial surfaces employed were glass slides (1 inch by 3 inches) coated with paraffin, except in one test where cellulose nitrate was substituted. A modification of the method of Evans and Martin (1935) was used in preparing these surfaces. After dipping the slides in liquid paraffin (M.P., 56° C.), maintained at a constant temperature of 75° C. by means of a water bath, they were placed in a vertical position until the paraffin cooled and solidified. Preliminary tests showed that the manner in which the slides were allowed to cool affected the thickness of the paraffin layer; and this in turn exerted an influence on the topography of the surface with a resultant variation in deposit. Consequently the slides were held in a constant temperature cabinet at 25° C. $\pm$0.5 and approximately 76 per cent relative humidity maintained by means of a saturated solution of sodium chloride during cooling and storage prior to use. Before analyzing for spray deposit, a strip of paraffin 2 mm. wide was removed from the margin of the slide to avoid variations which might result from handling the slides by the edges. For the cellulose nitrate surfaces, slides were dipped into 10 per cent cellulose nitrate dissolved in a mixture of 3 parts acetone and one part amyl acetate and then allowed to dry in a vertical position. Two tests were conducted using potato foliage as a surface.

APPARATUS.—A turntable, propelled by a small motor at a constant rate of eight revolutions per minute, supported the glass slides at an 85-degree angle 30 inches from the tip of a *No. 2 Weber Atomizer*. A baffle with a 2- by 3.75-inch aperture, which could be readily opened or closed, was imposed between the atomizer and turntable thus exposing the slides to spray only when passing directly in front of the atomizer. This arrangement permitted uniform deposition of materials on a series of replicate slides in the same operation. Insoluble particles of the spray mixture were kept in suspension by means of a mechanical agitator.

A *Coleman Model 10* spectrophotometer was used in the determination of copper.

PROCEDURE.—Three liters of spray were made up for each test run. In all sprays insoluble copper sulfate was used at the rate of 3 pounds per 100 gallons (10.8 g/3 l.); and, except where otherwise specified, wheat flour at the rate of 1 pound per 100 gallons (3.6 g/3 l.). In order

[1] The writers wish to acknowledge the technical assistance of Richard Hall, Willis Bruce and Glen Walsh.
[2] Tenacity as used here = the resistance of the spray deposit to weathering.
[3] $3Cu(OH)_2 - CuSO_4$

to insure maximum solution of the flour constituents, the flour was soaked overnight (3.6 gr. in 20 cc. solution) and made to volume with the other spray materials next day. After some preliminary experiments 10 revolutions of the turntable during the spraying process and 12 pounds pressure per square inch were selected as standard. The 10 revolutions in no case exceeded the point of "run off." Room temperature was held at approximately 25° C. Twelve slides arranged to form six pairs were placed on the turntable for each test run. After exposure to the spray the slides were dried. One from each pair of slides was selected at random and the initial deposit determined; and the remaining one of each pair was exposed to artificial weathering and the residual deposit determined.

ARTIFICIAL WEATHERING.—Weathering was simulated by spraying the slides with water, using the apparatus employed to apply the spray materials at 25 pounds pressure and a room temperature of approximately 25° C. It was found in a series of test runs that 150 turn-table revolutions for the washing operation, gave about 35 per cent retention of the standard, a desirable level for making comparisons with mixtures having a higher tenacity value. Accordingly 150 revolutions was set as standard for the washing (artificial weathering) operation. During the washing operation, which required about 19 minutes, the water in the spray tank was maintained at a constant level.

The length of time that the slides were allowed to remain in the drying cabinet before washing and the humidity and temperature of the drying cabinet were found to exert a significant influence on retention of the spray deposit. In preliminary tests, three sets of slides were sprayed with a mixture containing insoluble copper sulfate and then washed (25 lbs. per sq. in. and 100 revs.) at intervals of one, two, and three days, respectively. The mean tenacity index of the slides washed at an interval of one day was 24.4; two days, 48.0; and three days, 59.4. The two day period of time in a cabinet at 25° C. and 76 per cent relative humidity was used in subsequent tests.

TENACITY INDEX.—Tenacity of a spray mixture was determined through chemical analysis by comparing the washed slides with the unwashed slide of each pair. For each test run there were six paired values; to the mean of these the term "tenacity index" was assigned. The following definition of "tenacity index" is proposed:

$$T = \frac{RR}{IR} \times 100$$

Where T = Tenacity index

IR = Initial retention or quantity of material on a given area of a given surface immediately following application of the spray material, measured chemically.

RR = Residual retention or quantity of material on a duplicate area after weathering, either naturally or artificially.

"Tenacity index" differs from the "tenacity coefficient" of Heuberger (1940) in that it is based on actual quantities of materials present while tenacity coefficient is based on a biological assay using a fungus to measure the concentration of the toxicant. Tenacity index can be determined for any material; the tenacity coefficient is limited to those materials which inhibit spore development. Use of tenacity index provides a means for quickly and accurately evaluating the adhesive properties of a material. A weakness of the procedure is that values based on chemical assay are not necessarily indicative of values obtainable through biological tests.

Due to some unknown and uncontrolled factors the tenacity index of a material was found to vary from day to day. Quite possibly day to day humidity variations in the spray room may have been one of these factors. Accordingly data obtained on different days are not comparable.

LIVING SURFACES.—In the case of living material, plants were sprayed and samples taken before and after weathering. A given number of plants were treated by means of a compressed air sprayer and from each of these 20 to 30 subsamples of leaf surface units taken. "Before and after weathering" subsamples each consisted of one of a pair of oppositely-placed, equal-sized leaflets. A tenacity index was thus computed for each plant.

CHEMICAL ANALYSIS.—A modification of the method reported by Frear (1939) was employed to determine copper in the spray residues. Copper was dissolved from a slide by washing in a lipped petri dish with 15 ml. nitric acid solution (1:1 conc nitric). The solution was washed into a 200 ml. kohlrausch flask, and 30 ml. of

concentrated ammonium hydroxide and 10 ml. of a 20 per cent solution of ammonium citrate added, the latter to prevent iron[1] from forming a colored complex. One milliliter of a 1-per cent solution of sodium diethyl dithiocarbamate was added, the solution made to volume, mixed, and the density of the color of the solution determined immediately with the spectrophotometer at a wave length of 450 millimicrons.

A standard curve was prepared from percentage transmittance readings obtained from solutions of known concentrations of copper. It was found that concentrations ranging from 0.1 to 0.2 mg. per 100 ml. gave the most accurate readings. The results from a series of tests showed that digestion of the small amount of organic matter in the flour removed from the slides was not necessary.

When potato plants were used, leaf samples were collected in small jars and kept sealed in a refrigerator until ready to be used. They were then transferred to 300 ml. kjeldahl flasks and each jar rinsed, first with 15 ml. of 50 per cent nitric acid and then with distilled water. Ten milliliters of concentrated sulfuric acid were added to each flask and the leaves digested until all organic matter was detroyed and the digest perfectly clear. The digested sample was then transferred to a suitable volumetric flask (100, 200, or 500 ml., depending upon the concentration of copper) and filtered (discarding the first 50 ml.). A suitable aliquot was taken for copper analysis.

EXPERIMENTAL RESULTS AND DISCUSSION.—The term "standard spray mixture" as used hereafter means a mixture containing insoluble copper sulfate at the rate of three pounds per 100 gallons of water. Unless otherwise stated, tap water was used for making up the sprays, the slides were coated with paraffin, wheat flour was used at the rate of one pound per 100 gallons, and soybean flour at the rate of ¼ pound per 100 gallons.

In the first series of tests, the standard spray mixture was compared with mixtures containing flour that had been in contact overnight with various concentrations of acetic acid: 3.6 g. flour was dispersed in 20 cc. acetic acid solution and next morning made to 3 liter volume. The second test was a replicate of the first plus three additional combinations in which either lime or sodium hydroxide was substituted for acetic acid. The results of these tests (Table 1) may be summarized as follows:

[1] Trace of iron present in water.

Table 1.—The effect of wheat flour, wheat flour+acetic acid, wheat flour+lime and wheat flour+sodium hydroxide on the adherence of insoluble copper sulfate, on a paraffin surface.

MATERIALS[1]	TENACITY INDEX 1ST TEST	TENACITY INDEX 2ND TEST
Standard (3 lb. insoluble copper sulfate/100 gal.)	35	24
Standard+flour (1 lb./100 gal.)	68	50
Standard+flour soaked in N/500 acetic acid (final conc. n/52500)	69	48
Standard+flour soaked in N/100 acetic acid (final conc. n/15000)	60	49
Standard+flour soaked in N/50 acetic acid (final conc. n/7500)	64	58
Standard+flour soaked in N/25 acetic acid (final conc. n/3750)	72	54
Standard+flour soaked in N/10 acetic acid (final conc. n/1500)	76	66
Standard+flour soaked in lime (0.12 g.) water (final conc. 0.53 oz./100 gal.)		85
Standard+flour soaked in lime (1.8 g.) water (final conc. 8.0 oz./100 gal.)		52
Standard+flour soaked in N/10 sodium hydroxide (final conc. n/1500)		71

[1] Flour = wheat flour.

a. The addition of flour to the standard spray practically doubled the tenacity index value.
b. The addition of acetic acid to the flour had only slight, if any, effect.
c. The addition of lime to the flour produced a very marked increase in tenacity.
d. An excess of lime canceled the beneficial effect of smaller quantities.

It is of interest to note that, although the tenacity index value for a given combination varied between the first and second tests, the proportionate difference among the various combinations of the same date was maintained.

In the next two tests varying quantities of lime were added to the flour, mixed with 20 ml. water, allowed to remain overnight and then diluted to three liters. As shown in table 2, these results indicated that there was an optimum range for the amount of lime of 0.4 to 1.6 oz. per 100 gal. On the basis of these figures 0.24 g. was the amount selected to be used in further tests. When figured in terms of 100 gallons of spray, 0.24 g./3 liters is equivalent to approximately 1 ounce of lime per 100 gallons.

Nikitin & Anderson (1942) found that soybean flour was more tenacious than wheat flour. An experiment was designed to compare soybean flour with wheat flour

Table 2.—The optimum quantity of lime to use with wheat flour for maximum adherence of insoluble copper sulfate, on a paraffin surface.

Materials	Tenacity Index 1st Test	2nd Test
Standard (3 lbs. insoluble copper sulfate/100 gal.)	35	39
Standard+flour (1 lb./100 gal.)	42	43
Standard+flour+0.06 g. lime (final conc. 0.27 oz./100 gal.)	60	
Standard+flour+0.09 g. lime (final conc. 0.40 oz./100 gal.)	82	68
Standard+flour+0.12 g. lime (final conc. 0.53 oz./100 gal.)	81	80
Standard+flour+0.15 g. lime (final conc. 0.66 oz./100 gal.)	82	
Standard+flour+0.18 g. lime (final conc. 0.80 oz./100 gal.)	80	
Standard+flour+0.24 g. lime (final conc. 1.06 oz./100 gal.)	88	75
Standard+flour+0.30 g. lime (final conc. 1.33 oz./100 gal.)	82	
Standard+flour+0.36 g. lime (final conc. 1.59 oz./100 gal.)	80	71
Standard+flour+0.48 g. lime (final conc. 2.12 oz./100 gal.)	65	
Standard+flour+0.72 g. lime (final conc. 3.18 oz./100 gal.)	58	46

[1] Flour = wheat flour.

both with and without the addition of lime (Table 3). On the paraffin surface soybean flour alone imparted a greater degree of tenacity to the spray mixtures than did wheat flour alone. Upon the addition of lime, however, there was no difference in the tenacity index of the two materials.

Table 3.—A comparison of the effectiveness of wheat flour and soybean flour, alone and with lime added, on the adherence of insoluble copper sulfate, on a paraffin surface.

Materials	Tenacity Index
Standard (3 lb. insoluble copper sulfate/100 gal.)	39
Standard+wheat flour (1 lb./100 gal.)	43
Standard+soybean flour (4 oz./100 gal.)	65
Standard+wheat four+lime (1 oz./100 gal.)	75
Standard+soybean flour+lime	75

The results of an experiment designed to compare wheat flour and soybean flour on cellulose nitrate surfaces are summarized in table 4. The combination containing wheat flour alone appeared to be slightly superior to the one containing soybean flour alone, and the wheat flour-lime mixture was significantly superior to the soybean flour-lime mixture.

Preliminary investigations indicated that one pound of wheat flour per 100 gallons of mixture was a suitable concentration. However, the presence of lime might have an effect on the quantity of flour required for maximum adhesion. The results obtained by varying the quantity of flour are shown in table 5. Lime without flour was included in this series to demonstrate the effect of lime alone on the adhesive qualities of the spray. It seems that 8 oz. flour was not quite enough while all quantities above 8 oz. were equally effective.

Table 4.—A comparison of the effectiveness of wheat flour and soybean flour alone and with lime added on the adherence of insoluble copper sulfate, on a cellulose nitrate surface.

Materials	Tenacity Index 1st Series	2nd Series
Standard (3 lbs. insoluble copper sulfate/100 gal.)	16	14
Standard+wheat flour (1 lb./100 gal.)	24	23
Standard+soybean flour (4 oz./100 gal.)	17	17
Standard+wheat flour+lime (1 oz./100 gal.)	44	46
Standard+soybean flour+lime	23	23

Table 5.—The optimum quantity of wheat flour when lime is added to obtain maximum adherence of insoluble copper sulfate, on a paraffin surface.

Materials	Tenacity Index
Standard (3 lbs. insoluble copper sulfate/100 gal.)	33
Standard+wheat flour (8 oz.)+lime (1 oz./100 gal.)	82
Standard+wheat flour (12 oz.)+lime	94
Standard+wheat flour (16 oz.)+lime	96
Standard+wheat flour (20 oz.)+lime	95
Standard+wheat flour (24 oz.)+lime	95
Standard+lime	29

A concentration of soybean flour of 4 ounces per 100 gallons was based on the recommendation of Farrar & Flint (1938). Data given in table 6 indicate that there was little difference between 1 ounce and 8 ounces if lime was used with the soybean flour. A mixture containing 4 ounces soybean flour without lime was included to obtain additional data on the effect of lime on the adhesive properties of soybean flour.

Tap water was used in all of the preceding tests. In an attempt to determine if

Table 6.—The optimum quantity of soybean flour when lime is added to obtain maximum adherence of insoluble copper sulfate, on a paraffin surface.

MATERIALS	TENACITY INDEX
Standard (3 lbs. insoluble copper sulfate/100 gal.)	40
Standard+soybean flour (4 oz.)	55
Standard+soybean flour (1 oz.)+lime (1 oz./100 gal.)	80
Standard+soybean flour (2 oz.)+lime	82
Standard+soybean flour (4 oz.)+lime	87
Standard+soybean flour (6 oz.)+lime	80
Standard+soybean flour (8 oz.)+lime	83

the "hardness" of water affected the adhesive properties of the flour mixtures, two experiments were conducted (Table 7). These tests indicate that in the case of flour mixtures without lime the kind of water (tap or distilled) used has little effect on tenacity. With the flour-lime mixtures, the kind of water used in preparing the stock mixture which stood overnight with the flour and the water used in washing the slides was unimportant, but the kind of water used in diluting the spray was very important. In sprays where distilled water was used to dilute the stock mixture the presence of lime did not improve tenacity. Apparently the salts in the tap water were effective in producing greater tenacity than was obtained with distilled water.

Table 7.—The effect of "hardness of water" on the adhesive properties of wheat flour and flour plus lime, using insoluble copper sulfate as an indicator, on a paraffin surface.

MATERIALS[2]	TENACITY INDEX 1ST SERIES	TENACITY INDEX 2ND SERIES
Standard; S-T, W-T[1] (3 lbs. insoluble copper sulfate/100 gal. water)	33	24
Standard+flour; O-T, S-T, W-T (1 lb. wheat flour/100 gal.)	55	53
Standard+flour; O-D, S-T, W-T	57	44
Standard+flour; O-D, S-D, W-T	51	45
Standard+flour; O-D, S-D, W-D	60	65
Standard+flour+lime; O-T, S-T, W-T (1 oz. lime/100 gal.)	76	92
Standard+flour+lime; O-D, S-T, W-T	91	92
Standard+flour+lime; O-D, S-D, W-T	54	53
Standard+flour+lime; O-D, S-D, W-D	50	55

[1] O-T=over-night in tap water. O-D=over-night in distilled water. S-T=tap water used in making the spray mixture. S-D=distilled water used in making the spray mixture. W-T=washed with tap water. W-D=washed with distilled water.
[2] Flour=wheat flour.

Table 8 summarizes the results of a test to determine the components of flour responsible for its adhesive properties. The writers do not regard these tests as altogether conclusive, but there was an indication that this property can be largely attributed to the protein, perhaps to the gliadin fraction, although flour itself was much more effective than any flour constituent which was tried. This idea is in agreement with that reported by Nikitin *et al.* (1942).

Table 8.—The effect of the components of wheat flour, gliadin, gluten and starch, alone and with lime added on the adherence of insoluble copper sulfate, on a paraffin surface.

MATERIALS	TENACITY INDEX
Standard (3 lbs. insoluble copper sulfate/100 gal.)	38
Standard+wheat flour (1 lb./100 gal.)	50
Standard+wheat flour+lime (1 oz./100 gal.)	91
Standard+0.18 g. gliadin per 3 l.	47
Standard+0.36 g. gliadin per 3 l.	49
Standard+0.18 g. gliadin+lime	66
Standard+0.36 g. gliadin+lime	64
Standard+0.36 g. gluten	65
Standard+0.72 g. gluten	56
Standard+0.36 g. gluten+lime	63
Standard+0.72 g. gluten+lime	63
Standard+0.36 g. starch[1]	44
Standard+0.72 g. starch[1]	38
Standard+0.36 g. starch[1]+lime	27
Standard+0.72 g. starch[1]+lime	25

[1] Starch ball milled 7 days.

Two series of greenhouse experiments were conducted in which potted potato plants, arranged in blocks, were sprayed with the following mixtures[1] 1. (a) insoluble copper sulfate, (b) insoluble copper sulfate+flour, (c) insoluble copper sulfate +flour+lime; 2. (a) insoluble copper sulfate+flour+lime, (b) insoluble copper sulfate+soybean flour, (c) insoluble copper sulfate+soybean flour+lime. The results which are summarized in table 9 indicate that the flour-lime combinations possessed a greater degree of retention than any of the other mixtures.

SUMMARY.—Laboratory and greenhouse tests were conducted to determine the effect of wheat flour, both alone and in combination with certain other materials, upon the tenacity of spray components applied in the form of suspensions. Insoluble copper sulfate was used as the spray component or indicator, since its presence could be readily and accurately determined by chemical analysis.

[1] Insoluble copper sulfate 3 lb., flour 1 lb., lime 1 oz. and soybean flour 4 ounces per 100 gallons of spray.

Table 9.—The effect of wheat flour and soybean flour, alone and with lime added, on the adherence of insoluble copper sulfate on potato foliage, greenhouse tests.

Treatment	Block I	II	III	IV	V	VI	VII	VIII	Mean
	Tenacity Index								
Test 1									
Insoluble copper sulfate	33	51	35	14	22	41	29		32
Insoluble copper sulfate+wheat flour	35	45	46	37	35	43	35		39
Insoluble copper sulfate+wheat flour +lime	61	58	62	52	61	52	61		58
Minimum difference necessary for significance (P=0.05) 7.83									
Test 2									
Insoluble copper sulfate+wheat flour +lime	61	44	51	47	62	81	48	57	56
Insoluble copper sulfate+soybean flour+lime	46	53	47	38	39	38	37	52	44
Insoluble copper sulfate+soybean flour	47	45	44	37	37	41	36	49	41
Minimum difference necessary for significance (P=0.05) 8.18									

Wheat flour alone gave a significant increase in tenacity; and the addition of lime to the flour gave a significant increase over flour alone. The optimum amount of lime ranged from about 0.09 g. to 0.36 g. per 3 liters (equivalent to 0.4 to 1.6 oz. per 100 gal. spray). Smaller or greater quantities gave reduced values.

The addition of acetic acid or sodium hydroxide improved tenacity only slightly if at all.

On a paraffin surface, soybean flour alone was superior to wheat flour alone. With the addition of lime, however, they were equally effective. Although not conclusive, on a cellulose-nitrate surface there was an indication that soybean flour alone was slightly superior to the standard spray, and that wheat flour alone was superior to soybean flour alone. When combined with lime, wheat flour was superior to soybean flour on a cellulose nitrate surface.

When used with lime, the optimum quantity of wheat flour was found to be approximately 8 ounces or more per 100 gallons; and soybean flour, 1 ounce or more per 100 gallons.

Hardness of water had little effect on tenacity when flour was used alone, but in combination with lime the use of tap water in diluting the spray resulted in a markedly higher tenacity index than when distilled water was used.

Of the various components of flour, gliadin appeared to have the most effective adhesive properties.

Results of spray tests on greenhouse grown potato plants indicated: that the addition of flour alone increased the tenacity only slightly whereas the addition of flour plus lime markedly increased tenacity. The wheat flour-lime combination was significantly better than soybean flour alone or in combination with lime. The addition of lime to soybean flour increased tenacity only slightly if at all.—6-8-44.

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The Protective Value of Asphalt Laminated Paper against Certain Insects

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The need for pest and moisture-proof materials for wrapping and packaging foods, ammunition, and other products has led to the development of a number of new or little used materials. One of these wrappings consists of two or more layers of *Kraft* paper combined with asphalt as a laminating material. The asphalt is melted and applied to a layer of paper, then another layer of paper is pressed on the asphalt. Thus a wrapping material of two or more ply may be made. One adhesive tape used for sealing packages wrapped with asphalt laminated paper consists of strips of the laminated paper coated with an adhesive that is attached over the seams. This adhesive is about half and half bone glue and a petroleum asphalt emulsion. A solvent for the adhesive consists of a 15 per cent solution of equal parts by weight of *Glyoxol* (dialdehyde) and chrome alum. In use this tape is moistened with the solvent and applied to the package or box as a seal At the end of 72 hours a highly resistant water-proof and pest-proof bond is produced. Papers prepared by this and similar processes are being widely used in the present emergency (McCorison 1944).

Various organic fungicides may be added to the adhesive and to the laminated materials to resist fungous attack. The fungicide used in the asphalt-glue adhesive was *Dowicide G* (pentachlorophenol). The asphalt laminated paper was not treated with the fungicide.

Essig *et al.* (1943) have reported on the protective value of various wrapping materials, including asphalt laminated paper bags against a number of stored products pests, among which were species having well-known boring propensities such as the cadelle, *Tenebroides mauritanicus* and the lesser grain borer, *Rhizopertha dominica*. Such insects do not feed on paper but penetrate the bags for the food contained in them, for purposes of pupation, or because the bags happen to be in their way when boring. They did not test with insects such as roaches, thysanurans and termites, that frequently feed on paper for its food value. The roaches and thysanurans are surface feeders on paper and penetrate bags and wrappings by repeated feeding on the same areas rather than by boring. Much of the feeding of these surface feeders is for the finishes on and in the paper rather than the paper proper. The termites on the other hand prefer to feed within the food media and readily penetrate thin layers of paper, although they usually remain hidden if sufficient volume of paper is available.

The following species of insects were used in our tests:

Periplaneta americana	American cockroach
P. australasiae	Australian cockroach
Blatella germanica	German cockroach
Supella supellectilium	Brown-banded roach
Lepisma saccharina	Silverfish
Thermobia domestica	Firebrat
Ctenolepisma quadriseriata	Four-lined silverfish
Reticulitermes flavipes	Subterranean termite

The materials tested include the following:

2-ply asphalt laminated *Kraft* paper (30-30-30)
Raw *Solseal* adhesive tape with 0.15% of *Dowicide G.*
Raw *Solseal* adhesive tape with 0.30% of *Dowicide G.*
Raw *Solseal* adhesive tape with 1.00% of *Dowicide G.*
Solvent treated *Solseal* tape with 0.15% of *Dowicide G.*
Solvent treated *Solseal* tape with 0.30% of *Dowicide G.*
Solvent treated *Solseal* tape with 1.00% of *Dowicide G.*
Solseal tape treated and pressed onto asphalt laminated *Kraft* paper.

The tests were conducted in environments suitable for good activity of the various species of insects. The termites and roaches were kept at room temperature, 20° to 22° C., the silverfish at 27°, the four-lined silverfish at 30°, and the firebrat at 35° C.

The adhesive on the asphalt laminated paper is ready for use when treated with the solvent. Since this adhesive contains bone glue, a food relished by roaches and thysanurans, the raw adhesive was tested for attractiveness to these insects (Table 1). The adhesive tape containing 0.3 and 1.0 per cent of the fungicide was prepared only a few days preceding the tests, while

Table 1.—The attractiveness of raw asphalt-glue adhesive to roaches and thysanurans when offered with and without additional food. (Eighteen to 100 insects per sample.)

Insect	Additional Food	Duration of Test-Days	Per Cent Injury	
			Adhesive	Paper
	Containing 0.15 per cent *Dowicide*			
American roach	None	8	2.0	0.0
American roach	Bread	7	T	0.0
American roach	Bread	11	T	0.0
American roach	Bread	11	10.0	0.0
Banded roach	None	7	1.0	0.0
Banded roach	Cereal meal	7	23.0	0.0
Australian roach	Cereal meal	7	11.0	T
German roach	Cereal meal	11	5.0	0.0
4-lined silverfish	None	7	42.0	0.0
4-lined silverfish	None	9	12.0	0.0
4-lined silverfish	None	9	8.0	0.0
4-lined silverfish	Cereal meal	9	0.5	0.0
4-lined silverfish	Cereal meal	8	0.0	0.0
Firebrat	None	8	16.0	4.0
Silverfish	None	8	33.0	0.0
Silverfish	Cereal meal	7	T	0.0
	Containing 0.3 per cent *Dowicide*			
American roach	None	7	9.5	0.0
American roach	Bread	7	51.0	0.0
Banded roach	None	7	72.0	0.0
Banded roach	Cereal meal	7	75.0	0.0
4-lined silverfish	None	7	3.0	0.0
4-lined silverfish	Cereal meal	7	T	0.0
Firebrat	None	7	100.0	1.5
Firebrat	Cereal meal	7	98.0	0.0
Silverfish	None	7	57.0	0.0
Silverfish	Cereal meal	7	4.0	0.0
	Containing 1.0 per cent *Dowicide*			
American roach	None	7	0.5	0.0
American roach	Bread	7	0.05	0.0
Banded roach	None	7	0.0	0.0
Banded roach	Cereal meal	7	0.0	0.0
4-lined silverfish	None	7	0.38	0.0
4-lined silverfish	Cereal meal	7	0.1	0.0
Firebrat	None	7	0.75	0.2
Firebrat	Cereal meal	7	19.0	0.0
Silverfish	None	7	13.0	0.0
Silverfish	Cereal meal	7	0.5	0.0

the 0.15 per cent tape was from older stock. This may explain why the 0.3 per cent adhesive was more attractive to roaches and thysanurans than the adhesive containing less of the fungicide. It is evident that the glue in the adhesive rendered it an attractive and acceptable food, while the asphalt laminated paper was not attractive except to the firebrat. No evidence of repellency was observed. The addition of 1.0 per cent of the fungicide to the adhesive greatly reduced feeding by the roaches and four-lined silverfish, but did not render the adhesive sufficiently immune to attacks from the silverfish and firebrat to offer much protection.

Since the asphalt-glue adhesive is treated with a solvent before being applied to wrapped packages, the treated tape was tested for attractiveness to the insects. The treated tape was allowed to dry for 72 hours as recommended, before being tested (Table 2). It is evident that treating the adhesive with the solvent renders it much less attractive than when raw (Table 1) to roaches and thysanurans. The treated adhesive was relatively unattractive to roaches, the four-lined silverfish and silverfish, although slight feeding occurred. The firebrat, on the other hand, still fed freely on the adhesive and slightly damaged the paper.

The 2-ply asphalt laminated wrapping

Table 2.—The attractiveness of solvent treated asphalt-glue adhesive to roaches and thysanurans when offered with and without additional food. (Four to 100 insects per sample.)

Insect	Additional Food	Duration of Test-Days	Per Cent Injury	
			Adhesive	Paper
	Containing 0.15 per cent *Dowicide*			
American roach	None	8	T	0.0
American roach	Bread	7	0.0	0.0
Banded roach	None	7	0.0	0.0
Banded roach	Cereal meal	8	0.0	0.0
Australian roach	Cereal meal	7	T	0.0
4-lined silverfish	None	3	T	0.0
4-lined silverfish	Cereal meal	3	0.0	0.0
Firebrat	None	8	10.0	6.0
Silverfish	None	8	1.5	0.0
Silverfish	Cereal meal	7	T	0.0
	Containing 0.3 per cent *Dowicide*			
American roach	None	7	1.0	0.0
American roach	Bread	7	0.0	0.0
Banded roach	None	7	0.9	0.0
Banded roach	Cereal meal	7	T	0.0
4-lined silverfish	None	7	1.0	0.0
Firebrat	None	7	35.0	1.5
Firebrat	Cereal meal	7	11.0	0.0
Silverfish	None	7	2.0	0.0
Silverfish	Cereal meal	7	0.0	0.0
	Containing 1.0 per cent *Dowicide*			
American roach	None	7	0.6	0.0
American roach	Bread	7	0.0	0.0
Banded roach	None	7	0.0	0.0
Banded roach	Cereal meal	7	0.0	0.0
4-lined silverfish	None	7	0.0	0.0
4-lined silverfish	Cereal meal	7	T	0.0
Firebrat	None	7	15.0	1.5
Firebrat	Cereal meal	7	2.0	0.0
Silverfish	None	7	0.6	0.0
Silverfish	Cereal meal	7	0.01	0.0

paper was tested for resistance to the insects (Table 3). Damage to the surface and edge of the laminated paper is listed under "percentage injured," while complete penetration at edges or elsewhere is listed as "percentage destroyed." "Injury" did not penetrate the asphalt layer of material. Considerable resistance was shown to all species, but appreciable surface damage was produced by the American roach, four-lined silverfish and firebrat. The firebrat had demonstrated its ability to injure the paper in the previous tests (Tables 1 & 2). The four-lined silverfish in one test destroyed an area covering 1 per cent of the material on the edge of one sample. It appears that this type of protective wrapping would be of considerable value in protecting packaged materials from roaches and thysanurans, especially where other foods were available to the insects.

When the adhesive tape is treated with the solvent and pressed onto the asphalt laminated paper, only the latter is exposed to the insects except along the edges, especially if the tape is firmly adhered to the asphalt laminated wrapping. While the data in tables 1 and 2 give a measure of the attractiveness of the adhesive, raw and treated, and in table 3 the attractiveness of the asphalt laminated paper, they do not give a true value for the protective qualities to materials in sealed packages against these insects. The responses of these insects to tape tightly adhered to the asphalt laminated paper is shown in table 4. The adhesive on the tape used in this test contained 0.15 per cent of the fungicide. The firebrat was the only one of the seven species tested that produced an appreciable amount of surface injury. The asphalt layer in no sample had been reached by the feeding and there is no certainty that the firebrat or other species of thysanurans and roaches would pene-

Table 3.—The resistance of 2-ply asphalt laminated *Kraft* paper to roaches and thysanurans. (Four to 100 insects per sample.)

Insect	Additional Food	Duration of Test-Days	Per Cent Injured	Per Cent Destroyed
American roach	None	8	T	0.0
American roach	Bread	7	0.0	0.0
American roach	Bread	11	T	0.0
American roach	Bread	11	5.0	T
Banded roach	None	7	0.0	0.0
Banded roach	Cereal meal	7	0.0	0.0
Australian roach	Cereal meal	7	0.0	0.0
German roach	Cereal meal	11	0.0	0.0
4-lined silverfish	None	7	1.0	1.0
4-lined silverfish	None	9	T	0.0
4-lined silverfish	None	9	T	0.0
4-lined silverfish	Cereal meal	9	0.0	0.0
4-lined silverfish	Cereal meal	3	0.0	0.0
Firebrat	None	8	2.0	0.0
Silverfish	None	8	0.0	0.0
Silverfish	Cereal meal	7	0.0	0.0

trate this layer under usual conditions. Thus it appears that goods protected by wrapping with asphalt laminated paper sealed with a solvent treated asphalt-glue tape would be protected for considerable periods from roaches and thysanurans, except possibly the firebrat. Since the firebrat repeatedly injured the asphalt laminated paper (Tables 1 to 4) even when additional food was available, it could be expected to penetrate wrappings of this paper much more readily than the other species. Undoubtedly the availability of suitable foods other than the wrapping material would greatly influence the length of the protective period.

The termites are not surface feeders like the roaches and thysanurans and feed on paper for its food value rather than for the finishes used on the paper. The resistance of the various materials to termites is shown in table 5. The papers were placed where the termites would come into contact with them during normal activities. The soil contained wood on which the termites were feeding, thus starvation was not a factor in the tests. The insects were not attracted or repelled by the paper. They tubed over or chewed into the adhesive and paper depending on position in reference to their activities. This response was not unexpected as termites often tube over creosoted timbers. A slight amount of paper and sometimes asphalt was often destroyed in the process of tube building. There was no evidence

Table 4.—The resistance of *Solseal* tape attached to asphalt laminated *Kraft* paper to roaches and thysanurans. (Four to 100 insects per sample).

Insect	Additional Food	Length of Test-Days	Per Cent Injured	Per Cent Destroyed
American roach	None	8	T	0.0
American roach	Bread	7	0.0	0.0
American roach	Bread	9	0.0	0.0
American roach	Bread	9	T	0.0
Banded roach	None	7	0.0	0.0
Banded roach	Cereal meal	7	T	T
Australian roach	Cereal meal	7	0.0	0.0
German roach	Cereal meal	9	0.0	0.0
4-lined silverfish	None	7	0.0	0.0
4-lined silverfish	None	7	T	0.0
4-lined silverfish	None	7	T	0.0
4-lined silverfish	Cereal meal	7	T	0.0
4-lined silverfish	Cereal meal	3	0.0	0.0
Firebrat	None	8	12.0	0.05
Silverfish	None	8	T	0.0
Silverfish	Cereal meal	7	0.0	0.0

Table 5.—The resistance of asphalt laminated paper and an asphalt-glue adhesive tape to termites.

No. of Insects	Length of Test Days	Test Material	Dowacide Per Cent	Adhesive Per Cent	Asphalt Paper	
					Per Cent Injured	Per Cent Destroyed
32	14	Raw adhesive	0.15	0.0	—	0.0
100	21	Raw adhesive	0.15	12.0	—	2.0
32	22	Raw adhesive	0.30	0.0	—	0.0
100	22	Raw adhesive	0.30	0.0	—	0.0
32	22	Raw adhesive	1.00	0.0	—	0.0
100	22	Raw adhesive	1.00	0.0	—	0.0
32	14	Treated adhesive	0.15	0.0	—	0.0
100	21	Treated adhesive	0.15	0.25	—	0.0
32	22	Treated adhesive	0.30	0.0	—	0.0
100	22	Treated adhesive	0.30	0.0	—	0.0
32	22	Treated adhesive	1.00	0.0	—	0.0
100	22	Treated adhesive	1.00	0.0	—	0.0
32	14	Laminated paper	None	—	2.0	0.0
100	21	Laminated paper	None	—	2.0	1.5
32	14	Tape and paper	None	—	0.0	0.0
100	21	Tape and paper	None	—	10.0	0.0

that feeding on the paper or adhesive occurred. The samples containing 0.3 and 1.0 per cent of the fungicide were uninjured.

The fungicide, *Dowicide G*, in the asphalt-glue adhesive did not offer adequate protection against fungous attack with strengths up to 1.0 per cent. *Chaetomium globosum*, a common fungus attacking paper, developed on all samples where the relative humidity was sufficient to promote growth. *Aspergillus* sp. and *Penicillium* sp. were common in the cultures. Two unidentified species of fungi, one probably parasitic on *Chaetomium*, were found on several samples.[1]

SUMMARY.—A 2-ply asphalt laminated *Kraft* paper offered considerable resistance to penetration by four species of roaches and three species of thysanurans. The American roach and firebrat produced the most damage. A petroleum asphalt emulsion and glue adhesive applied to strips of the laminated *Kraft* used for sealing the wrappings formed an attractive food to the roaches and thysanurans in the raw state, but was much less attractive after treatment with a solvent. Only the laminated *Kraft* surfaces are exposed to the insects on sealed packages.

Termites tubed over the laminated *Kraft* paper and penetrated it at times, but the asphalt layer seemed to offer some resistance to penetration.

A fungicide, *Dowicide G*, in the adhesive offered little protection against fungi in moist environments. A 1 per cent concentration of the fungicide greatly reduced the attractiveness of the adhesive to roaches and thysanurans, but the firebrat and silverfish still fed extensively on the adhesive during a 7-day test. Termites tubed over, but did not injure paper in contact with 0.3 and 1.0 per cent of fungicide in the adhesive, but did damage paper in contact with weaker concentrations of the fungicide.

CONCLUSION.—A 2-ply asphalt laminated *Kraft* paper sealed with an asphalt-glue adhesive offered considerable resistance to feeding and penetration by four species of roaches (American, Australian, German, and brown-banded), three species of thysanurans (silverfish, firebrat, and four-lined silverfish), and some resistance to a subterranean termite. The termites and firebrat seemed most likely to penetrate the wrapping paper in the shortest period of time.—6-23-44.

[1] All identifications of fungi were made by Dr. Thomas Sproston of the Botany Department, Massachusetts State College.

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The Use of Oleic Acid and Aluminum Sulfate to Increase Deposits of Nicotine Bentonite

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Various combinations of nicotine have been used at Yakima, Wash., in the search for nonarsénical materials for the control of the codling moth, *Carpocapsa pomonella* (L.), under the arid conditions in the Pacific Northwest. Tests with tank-mix nicotine bentonite, made in this area by Dean *et al.* (1942), indicated that this combination was unsatisfactory because of the objectionable residue left on the fruit at harvest and because of the loss of protection against worm attack late in the season. McCrory & Vinson (1938) and Vinson & McCrory (1940) pointed out that by mixing the nicotine and bentonite and adding this combination to the tank in a dry form the objectionable residue could be avoided; and in 1940 the Vincennes, Ind., laboratory of the Bureau of Entomology and Plant Quarantine tested this combination with the addition of soybean oil with good results.[2] In 1941, therefore, the dry-mix nicotine bentonite with soybean oil was tested in the field at Yakima. The results that season were not very promising and in 1942 and 1943 a method of preparation was developed which gave a much better mixture for use under the conditions existing in the Pacific Northwest, and a discussion of results with this method is presented in this paper.

Methods.—Field experiments were conducted in three orchards in 1941 and 1942 and in two orchards in 1943. The following apple varieties were used: Orchard A, Winesap; orchard B, Rome; and orchard C, Delicious. The experimental block in each orchard was divided into eight parts, and each of a number of spray treatments was applied to one tree in each part. Thus each treatment was replicated in eight single-tree plots in each orchard. A number of treatments other than those described in this paper were also applied in these experiments. Except in orchard A in 1943, when a part of the block was injured by frosts, the size of the crop was uniform within orchards for each year, and apparently it was not influenced by the treatments. Orchard B was divided into two sections in 1943, each containing a series of replicated treatments. The spraying was done with a stationary power sprayer with a limited extension of pipe lines, at a pressure of about 350 pounds per square inch. A calyx spray of lead arsenate was applied to all plots, and the test materials were used in the cover sprays, which were applied as often as necessary for adequate control.

Examinations of 250 apples, taken at random from the entire crop, including drops, were made at harvest from each tree. The data were subjected to analysis of variance. Between means in various groups, differences required to indicate significance at odds of 19 to 1 were computed.

Chemical analyses of spray deposits were made on samples of apples taken immediately before and immediately after each spray application. The method of sampling and the method of preparing the samples were described by Dean *et al.* (1942). The colorimetric method of nicotine analysis described by Markwood (1939) and the method of arsenic analysis described by Cassil & Wichmann (1939) were used.

A standard lead arsenate spray containing mineral oil and a deposit builder was included in each experiment. Average deposits of 30 to 40 micrograms of As_2O_3 per square centimeter of apple surface were found on the fruit after spraying.

In the laboratory experiments small, mature Winesap apples were used in practically all instances. At least 5 sprayed apples were used for the chemical analyses. Each apple to be sprayed was impaled upon a wire spike which was set in a rotating spindle making about 1 revolution per second. The nozzle of the sprayer was 44 inches from the apple being sprayed, and a pressure of about 300 pounds per square inch was maintained during the

[1] At the time this work was done Mr. Cassil was a chemist in the Bureau of Entomology and Plant Quarantine. He has since resigned.

[2] Unpublished reports. The help of the staff of the Vincennes laboratory in preparing much of the dry-mix material used at Yakima is acknowledged.

application. Although only 3 gallons of spray was prepared for a test, the quantities of materials given in this paper are for 100 gallons of spray.

Apples carrying some of the spray mixtures giving the better deposits were subjected to larvicide tests, 6 to 8 codling moth eggs in the blackspot stage being attached to each apple, and the apples being examined for worm injury about a week later. From 10 to 30 apples were used for the larvicide test of each of these mixtures. Unsprayed apples were used as checks. About 85 per cent of the eggs used in laboratory tests hatched, and about 76 per cent of the resulting larvae on unsprayed apples entered these fruits. Because of variations, probably due to the small numbers of larvae used in the larvicide tests, the results were considered only as indications of what could be expected in the field.

FIELD EXPERIMENTS IN 1941.—A tank-mix nicotine bentonite, used as in previous work (Dean *et al.*, 1942), was again unsatisfactory and a dry-mix material gave even poorer results. Chemical analyses showed that the dry-mix material deposited less nicotine than the tank-mix. The deposit from the dry-mix nicotine bentonite was uneven and spotty, and it was believed that a method of application could be devised that would give a higher deposit of nicotine and a smoother cover with reduced dosages of material.

LABORATORY EXPERIMENTS IN 1941 AND 1942.—During the winter numerous sprays containing dry-mix nicotine bentonite were prepared and applied to apples. The most promising of these consisted of 3 pounds of nicotine bentonite, 1 quart of emulsible summer oil, and 1 ounce of aluminum sulfate[3] per 100 gallons of spray. These materials could be put directly into the tank without preliminary manipulation. This spray gave a deposit of about 6 micrograms of nicotine per square centimeter of apple surface, which was about the same deposit as given by a spray containing 6 pounds of nicotine bentonite applied with soybean oil. Larvicide tests indicated, however, that the spray containing 3 pounds of nicotine bentonite was more efficient, probably because it gave a smoother and more even deposit.

[3] Commercial grade; a mixture of $Al_2(SO_4)_3 \cdot 9H_2O$ and $Al_2(SO_4)_3 \cdot 18H_2O$, averaging about $14 \cdot 5H_2O$.

When bentonite designated by the manufacturer to be 300 mesh was used in preparing nicotine bentonite, the resulting material was rather coarse and grainy. Material that had been passed through a flour sifter had a surface mean particle diameter of 13.7 microns. In order to obtain material with a smaller particle size, a small air separator was constructed, and through its use material having a surface mean particle diameter of 10.3 microns was obtained. Little difference in efficiency could be demonstrated between materials of these two particle sizes, but it was believed that if the size could be reduced further its efficiency would be increased. Both materials contained about 8.5 per cent of nicotine.

FIELD EXPERIMENTS IN 1942.—The nicotine bentonite used for field tests in 1942 was prepared by mixing 40 per cent nicotine sulfate and bentonite and then putting this mixture through a micronizer. The final product contained 10 per cent of nicotine and had a surface mean particle diameter of 6.3 microns. A spray containing 3 pounds of this micronized nicotine bentonite, 1 quart of emulsible summer oil, and 1 ounce of aluminum sulfate per 100 gallons was applied, six cover sprays being used in orchards A and B and four cover sprays in orchard C. Although this spray did not give so smooth a cover in the field as it did in the laboratory, it was better than the dry-mix nicotine bentonite spray applied in 1941.

The average deposit on apples from the Winesap and Rome orchards was 6.8 micrograms per square centimeter immediately after spraying and 3.2 micrograms at the close of the between-spray periods. These deposits of nicotine were considerably less than those obtained in 1941, when about 11 micrograms per square centimeter was found. Analyses of deposits were not made in the Delicious orchard in 1942.

The examinations of fruit at harvest-time showed that, although the nicotine spray allowed more worms per 100 apples than the lead arsenate in all orchards, the difference was statistically significant only in orchard A (Table 1). This was a definite improvement over the results obtained in 1941, when twice the amount of nicotine bentonite produced no better results.

LABORATORY EXPERIMENTS IN 1942–43.

Table 1.—Comparative efficiency of micronized dry-mix 1:5 nicotine bentonite and lead arsenate in controlling the codling moth in the field, 1942.

Treatment (Quantity per 100 Gallons)	Number of Worms or Injuries per 100 Apples					
	Orchard A		Orchard B		Orchard C	
	Worms	Injuries	Worms	Injuries	Worms	Injuries
Lead arsenate 3 lbs., spreader ½ lb., emulsible oil 1 qt.[1]	6.5	29.7	11.0	34.4	14.2	48.1
Nicotine bentonite 3 lbs., oil emulsion 1 qt., aluminum sulfate 1 oz.	21.0	32.6	20.7	30.9	21.6	34.8
Difference required for significance at odds of 19 to 1	8.0	16.8	19.7	32.7	13.1	27.8

[1] Two quarts of oil were used in ovicidal sprays.

—Since the field experiments in 1942 had shown that, by changing the method of preparation, the efficiency of dry-mix nicotine bentonite could be improved, about 600 nicotine sprays were prepared, applied to apples, and analyzed for nicotine deposit during the winter.

In the Pacific Northwest fatty acids are frequently used with metallic salts and mineral oils to get good deposits of lead arsenate from codling moth sprays. Naphthenic, stearic, palmitic, and oleic acids were therefore compared in various mixtures. Although the first three have possibilities, oleic acid, which had shown promise the previous winter, gave better deposits, and the material is easily available. It was therefore used in most of the mixtures tested in 1942–43. Of two metallic salts tried, aluminum sulfate gave higher deposits than zinc sulfate. In work with deposit-building nicotine bentonite sprays Steiner *et al.* (1943) pointed out that free fatty acids found in crude soybean phosphatides may play an important rôle.

The usual procedure in mixing these sprays was as follows: Three pounds of nicotine bentonite was stirred into about 2 gallons of water in a bucket; the mineral oil containing the oleic acid was then added and mixed well with a wire beater. The aluminum sulfate dissolved in a little water was then added, and the mixture was either poured or injected into the spray tank. A summer mineral oil having a Saybolt viscosity of 60–64 seconds at 100° F. and an unsulfonated residue of 79 per cent was used in most of these tests, although commercial emulsible oils with similar base oils could probably have been used.

Since in a spray that portion of nicotine which is soluble in the water may be largely lost in the run-off, the water solubility of nicotine was studied in nearly all the mixtures used. This was done by determining the nicotine content of the filtrate of the spray mixture, Neither the proportion of oleic acid nor that of the oil used had an appreciable effect on the percentage of water-soluble nicotine in a spray. But, as shown in table 2, when no aluminum sulfate was included in sprays containing 3 pounds of 1:5 nicotine bentonite, about 12.5 per cent of the nicotine was dissolved in the water; when 2 ounces of aluminum sulfate was added, about 20 per cent of the nicotine was dissolved in the water; and when 1 pound of this salt was added, about 36 per cent was dissolved. By doubling the quantity of nicotine bentonite the percentage of the total nicotine dissolved was reduced by about one-third; obviously this corresponds to an increase of about one-third in the quantity of nicotine dissolved.

In order to study further the water solubility of nicotine, a nicotine bentonite containing 1 pint of 40 per cent nicotine sulfate and 10 pounds of bentonite was prepared. This material will hereafter be referred to as 1:10 nicotine bentonite. The results given in table 3 show that, when 4 pounds of the 1:10 material was used, less than 6 per cent of the total nicotine was water soluble. After deduction of the water-soluble nicotine there is about the same percentage of insoluble nicotine in a spray made with 3 pounds

Table 2.—Nicotine deposits and water-soluble nicotine from dry-mix 1:5 nicotine bentonite sprays for the codling moth. Laboratory tests, 1942–43. (Quantities of materials are for 100 gallons of spray.)

Oleic Acid, Pints	Aluminum Sulfate, Ounces	Nicotine Bentonite 3 Pounds: Nicotine Deposit, Micrograms per Square Centimeter, after 3 Seconds	Nicotine Bentonite 3 Pounds: Nicotine Deposit, Micrograms per Square Centimeter, after 6 Seconds	Nicotine Bentonite 3 Pounds: Water Soluble Nicotine, per cent	Nicotine Bentonite 6 Pounds: Nicotine Deposit, Micrograms per Square Centimeter, after 3 Seconds	Nicotine Bentonite 6 Pounds: Nicotine Deposit, Micrograms per Square Centimeter, after 6 Seconds	Nicotine Bentonite 6 Pounds: Water Soluble Nicotine, per cent
0	0	4.8	5.2	12.4	—	—	—
0.5	2	15.9	28.6	19.7	19.3	30.5	12.1
0.5	4	11.4	18.5	26.8	24.3	39.6	17.6
1	0	5.5	4.4	12.5	—	—	—
1	2	18.2	36.0	20.7	25.2	34.6	13.8
1	4	15.0	24.7	28.9	30.3	45.2	18.1
1	8	19.8	27.8	33.7	24.3	34.6	22.8
1	16	16.6	31.3	35.9	26.7	39.0	23.5
2	8	18.5	27.8	34.7	—	—	—
4	16	23.6	34.1	36.0	—	—	—

[1] 2 quarts of mineral oil were used in all sprays.

of the 1:5 material as in one made with 4 pounds of the 1:10 material.

The reaction between the oleic acid, the aluminum sulfate, and the other materials is not understood, but increased deposits could be obtained only by adding both aluminum sulfate and oleic acid. Without the sulfate, merely increasing the spray exposure from 3 seconds to 6 seconds did not appreciably increase the deposit (Table 2). With all mixtures containing the two materials, however, a definite increase in deposit resulted when the spraying time was doubled. When either 1 pint or 0.5 pint of oleic acid was used, the best deposits of nicotine were obtained by adding aluminum sulfate at the rate of 2 ounces to each 3 pounds of nicotine bentonite used.

The sprays listed in table 3 were applied after an injector had been attached to the laboratory sprayer. This injector which was similar to one used on the field sprayer, worked on the suction principle of a tank filler. Through its use the slurry of spray materials was forcibly mixed with water in the tank. Injected sprays gave higher deposits in the laboratory than those not injected.

When sprays containing 1:10 nicotine bentonite were prepared in the same manner as those containing the 1:5 material, a light deposit resulted. It was found that by mixing the nicotine bentonite in oil and oleic acid before adding the water and aluminum sulfate good deposits could be obtained. Visual observations indicated that the quantity of nicotine in in the 1: 10 material was not enough to prevent the swelling of the bentonite, and when swelling occurred a good deposit

Table 3.—Comparison of nicotine deposits and water-soluble nicotine from 1:5 and 1:10 nicotine bentonite sprays for the codling moth. Laboratory tests, 1942–43. (Quantities of materials are for 100 gallons of spray.)

Nicotine Bentonite, Pounds	Mineral Oil, Quarts	Oleic Acid, Pints	Aluminum Sulfate, Ounces	Nicotine Deposit, Micrograms per Square Centimeter, After 3 Seconds	Nicotine Deposit, Micrograms per Square Centimeter, After 6 Seconds	Water Soluble Nicotine, Per Cent
1:5 Nicotine Bentonite (Nicotine 9.3%)						
3	2	1	4	24.2	37.0	31.7
	1	1	4	24.9	37.8	36.5
	2	0.5	2	22.1	38.7	25.7
	1	0.5	2	20.3	29.3	27.4
1:10 Nicotine Bentonite (Nicotine 4.8%)						
4	2	1	2	5.4	6.8	2.3
			4	18.0	26.7	4.6
			8	22.4	37.5	5.4

Table 4.—Nicotine deposits on apples in orchards sprayed with dry-mix nicotine bentonite or the codling moth. 1943.

Time of Analysis (Before or After Spray)	Nicotine, Micrograms Per Square Centimeter of Apple Surface			
	Orchard A		Orchard B	
	Nicotine Bentonite 1:5	Nicotine Bentonite 1:10	Nicotine Bentonite 1:5	Nicotine Bentonite 1:10
After 1st	8.2	6.3	6.6	7.1
Before 2d	3.5	3.5	3.7	4.3
After 2d	10.5	6.0	10.9	9.7
Before 3d	3.9	3.5	5.3	4.3
After 3d	9.8	6.6	9.6	6.9
Before 4th	6.5	4.5	7.0	5.0
After 4th	11.8	8.7	13.1	9.3
Before 5th	4.6	4.5	5.1	3.6
After 5th	10.1	6.8	11.3	5.9
Before 6th	6.6	4.0	7.4	2.6
After 6th	9.3	5.9	12.1	3.7
Before 7th	6.7	5.1	—	—
After 7th	9.4	8.3	—	—
Harvest	5.8	5.5	7.1	1.5
Average before	5.3	4.2	5.7	4.0
Average after	9.9	6.9	10.5	7.1

was not obtained. This agrees with the findings of Steiner *et al.* (1943).

Tests against larvae indicated that 1:5 nicotine bentonite was more efficient when applied with mineral oil, oleic acid, and aluminum sulfate than when applied with emulsible oil and aluminum sulfate, as used in the field tests in 1942. There was no difference in efficiency between 3 pounds of 1:5 nicotine bentonite and 4 pounds of the 1:10 material.

FIELD EXPERIMENTS IN 1943.—A spray containing 3 pounds of 1:5 nicotine bentonite was compared in field tests with one containing 4 pounds of the 1:10 material. The 1:5 material contained 8.1 per cent of nicotine and had a surface mean particle diameter of 3.9 microns, whereas the 1:10 material contained 4.3 per cent of nicotine and had a surface mean particle diameter of 6.9 microns.

When the field spraying had been begun it was found that adjustments had to be made in the quantities of supplemental materials because of the difference between the irrigation water used in field spraying and the tap water used in the laboratory. Commercial oleic acid, or red oil, was used in the field in place of the C. P. oleic acid used in the laboratory, and this also necessitated some changes.

In the first cover spray with 1:5 nicotine bentonite 1 pint of oleic acid was used with 2 ounces of aluminum sulfate in both orchards. This quantity of oleic acid was found to be excessive, and in later sprays it was reduced to one-fourth pint. A better type of deposit resulted when the oleic acid and aluminum sulfate were mixed with the dry nicotine bentonite before they were added to the water. It was found that this should be done at least a day before the spray is applied. Just before application this mixture was added to several gallons of water followed by 1 or 2 quarts of mineral oil, and then this slurry was injected into the spray tank.

Good deposits were not consistently obtained with the 1:10 nicotine bentonite in the field, although various proportions of supplemental materials and methods of mixing were tried. One-third pint of oleic acid and 2 ounces of aluminum sul-

fate were used with 4 pounds of the 1:10 nicotine bentonite in most of the sprays. In the last spray in orchard A (Table 4) the aluminum sulfate was dissolved in water in a bucket before the nicotine bentonite -oleic acid mixture was added. The oil was then added to the slurry before it was injected into the tank. This method of mixing apparently stopped the swelling of the bentonite and gave a good deposit.

As shown in table 4, about 10 micrograms of nicotine per square centimeter of apple surface was found in the 1:5 nicotine bentonite plots after spraying, while about 7 micrograms was found in the 1:10 plots. After all the sprays had been applied, the deposits appeared to be rather heavy and uniform, particularly after the last application on the Winesap apples (Fig. 1).

At harvest, apples sprayed with the 1:5 material were put through washing machines such as are regularly used for removing deposits of lead arsenate. The following washes satisfactorily removed the visible residue: (1) Soda ash, 80 pounds per 100 gallons of water at 90° F., followed by a 1.5 per cent cold hydrochloric acid bath and a water rinse; (2) sodium silicate, 80 pounds per 100 gallons of water at 105°, followed by a 1.5 per cent hydrochloric acid bath at 85° and a water rinse. Somewhat simpler washes might be equally effective, although a single cold acid bath did not satisfactorily clean the fruit.

The experiments as a whole (Table 5) showed that no significant difference in the control of the codling moth was afforded between the 1:5 nicotine bentonite and the lead arsenate in either worms or injuries per 100 apples except in the case of injuries in orchard A. The 1:10 nicotine bentonite gave significantly poorer control than lead arsenate in orchard B; in orchard A there was no difference in worm control and the injuries were significantly fewer where the nicotine was used. There was no significant difference between the 1:10 nicotine bentonite and the 1:5 nicotine bentonite except in section 1 of orchard B, where results from the former were poorer.

WEATHER DURING THE PERIOD OF THE EXPERIMENTS.[1]—Rainfall in the arid Yakima Valley is light, the normal annual precipitation being 6.78 inches. For the months of May to September, inclusive, the period during which most protection from worm attack is needed, normal rainfall is 2.22 inches. The actual rainfall for these months during the years covered by this work was less than normal, being lowest in 1943 and highest in 1941, and it did not greatly exceed the normal in any one month.

The temperature was generally similar during the three seasons. During the last week of August and through September in 1941 the temperature was slightly below normal and there was practically no codling moth activity, but during September in 1942 and 1943 the temperature was above normal and codling moths re-

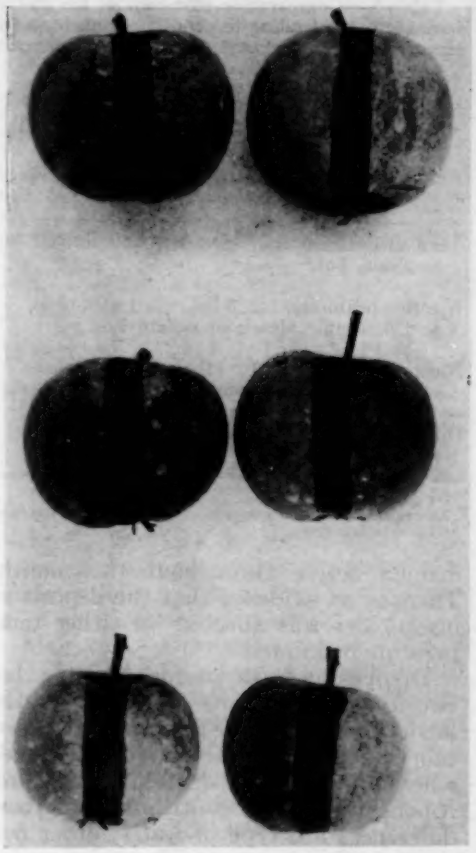

FIG. 1.—Winesap apples from field spray experiments on August 25, 1943, after 7th cover spray. Top to bottom, lead arsenate, nicotine bentonite 1:5 and nicotine bentonite 1:10. A strip of residue was removed from each apple to show contrast.

[1] All data were taken from the U. S. Weather Bureau Station, Yakima, Wash.

Table 5.—Comparative efficiency of 1:5 and 1:10 micronized dry-mix nicotine bentonite and lead arsenate in controlling the codling moth in the field. Yakima, Wash., 1943.

Treatment (Quantities per 100 Gallons)	Number of Worms or Injuries per 100 Apples					
	Orchard A		Orchard B			
			Section 1		*Section 2*	
	Worms	Injuries	Worms	Injuries	Worms	Injuries
Lead arsenate, 3 lbs.; spreader, 0.33 lb.; oil emulsion, 1 qt.[1]	66.7	224.1	6.2	12.5	9.9	27.1
Nicotine bentonite, 1:5, 3 lbs.; oil, 1 qt.[2]; oleic acid, 0.25 pint[2]; aluminum sulfate, 2 oz.	47.0	74.8	6.3	9.4	31.4	42.2
Nicotine bentonite, 1:10, 4 lbs., oil, 1 qt.[2]; oleic acid, 0.33 pint; aluminum sulfate, 2 oz.	61.4	99.2	27.6	34.6	56.5	69.9
Differences required for significance at odds of 19 to 1	21.7	62.8	14.1	18.0	27.8	42.2

[1] 2 quarts in ovicide sprays.
[2] 2 quarts in first three sprays in all orchards and in last spray in orchard A.
[3] 1 pint in first spray.

mained active throughout the month. There is no evidence that the deposit of insecticides was affected by either temperature or rainfall.

DISCUSSION.—Variations found between spray deposits obtained in the laboratory and in the field show that certain mixtures must be carefully adjusted when applied under different conditions. Among the factors contributing to these differences are type of water, order and method of mixing materials, and spraying equipment. Even though these differences occur, laboratory work is useful in indicating types of spray mixtures to be used in the field.

As pointed out by Dean *et al.* (1942), the number of worms per 100 apples is a more important criterion in evaluating results than the total number of injuries. Stings, unless there are more than 4 or 5 per apple, do not place the fruit in the cull class, whereas 1 worm in an apple produces a cull, thus greatly reducing the market value. On this basis the dry-mix nicotine bentonite spray equaled the standard lead arsenate spray in all experiments in 1943 only.

Since micronized and nonmicronized materials were not compared directly in the field, the importance of particle size is unknown. Deposits of 11 micrograms of nicotine per square centimeter of apple surface in 1941 were no more efficient than deposits of 6.8 and 10.0 micrograms in 1942 and 1943, respectively. The materials used in 1942 and 1943 were micronized, whereas that used in 1941 was not. Both particle size and better type of cover undoubtedly contributed to the efficiency of the 1:5 nicotine bentonite used in 1943.

Calculated at prices in effect in 1943, the 1:5 nicotine bentonite spray costs about 65 cents per 100 gallons, and the standard lead arsenate spray costs about 50 cents. By improving the spray the amount of 1:5 nicotine bentonite used in the mixture was reduced from 6 pounds in 1941 to 3 pounds in 1943, while at the same time the efficiency in controlling the codling moth was increased to that of lead arsenate. Thus the cost of materials has been cut in half.

CONCLUSIONS.—Results of tests reported in this paper indicate that 1:5 dry-mix nicotine bentonite applied with mineral oil, oleic acid, and aluminum sulfate is as efficient as lead arsenate in controlling the codling moth in the Pacific Northwest. The amount of nicotine sulfate used in these sprays is half that used in tank-mix nicotine bentonite sprays. Washes used for cleaning apples sprayed with lead arsenate satisfactorily removed the spray deposit at harvest. As used, 1:10 nicotine bentonite did not control the codling moth. Until the amount of nicotine needed in sprays is reduced or the price of nicotine lowered, nicotine bentonite sprays are not so economical as lead arsenate for controlling the codling moth in the Pacific Northwest.—7-3-44.

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Repellency of Pyrethrum Extract and Other Materials to Full-Grown Codling Moth Larvae

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Chemically treated bands are only partially effective in codling moth control, because a considerable proportion of the codling moth larvae on an apple tree cocoon elsewhere than under or in the bands. Tests were begun in 1936 at the Yakima, Wash., laboratory to develop ways of inducing a larger proportion of the larvae to enter the bands, by applying a repellent to the trunks and scaffold limbs of apple trees. This work has shown that certain pyrethrum combinations are repellent to codling moth larvae, and it remains to be determined whether these repellent materials, when applied to the trees, will induce a greater proportion of the mature larvae to enter the bands.

In 1931 Rolfs[1] found that bands impregnated with either of two proprietary preparations of pyrethrum, one diluted 50–50 with red engine mineral oil and the other undiluted, repelled 97 to 98 per cent of full-grown codling moth larvae when placed on badly infested apple-tree trunks, and that such bands were repellent for at least 3½ months. The red engine oil showed no repellency when tested without the pyrethrum extract.

Gradinger *et al.* (1940) reported that pyrethrum extract, when applied to apple trees in July at the rate of 60 mg. of pyrethrins per 100 cc. of kerosene, showed no permanent repellent action to mature codling moth larvae. However, of some 250 formulas tested by the present writers in the laboratory and in several orchards over a period of 8 years, only pyrethrum extract, alone and in combination with cottonseed oil or kerosene, showed worthwhile repellency, and this paper deals chiefly with these materials.

METHODS.—Corrugated-paper strips, 2 inches wide, were wound in small rolls and soaked in the test chemicals for 60 seconds. The strips were allowed to dry a day or two and were then placed as bands half way around apple-tree trunks, where they were opposed by similar untreated half bands. The bands were usually applied early in July after fair numbers of larvae had started to leave the fruit. In most instances each material was tested on 3 to 9 trees. These bands were not shifted during the test because some of the test material might be retained on the bark where the repellent band had been, and thus influence the results. Usually, however, the position of the treated and untreated half bands was varied from tree to tree with respect to the points of the compass. Frequent counts were made, usually weekly, of the empty pupal cases protruding from the upper and lower edges of the bands. At the final examination, when the bands were removed and the numbers of larvae were recorded, the cocoons from which these protruding cases had come were not recounted. If the treated half bands caught more larvae than the untreated halves, the material with which they were treated was considered to be attractive rather than repellent.

The method of determining the percentage of repellency may be illustrated

[1] A. R. Rolfs, unpublished notes, Bureau of Entomology and Plant Quarantine laboratory, Yakima, Wash.

Table 1.—Repellency of three undiluted materials to mature codling moth larvae. Yakima, Wash.

Material	Larvae Caught in Bands and Repellency of Materials after Exposure of—								
	30 Days			60 Days			90 Days		
	Larvae in Bands		Repellency	Larvae in Bands		Repellency	Larvae in Bands		Repellency
	Treated	Total		Treated	Total		Treated	Total	
	Number	*Number*	*Per Cent*	*Number*	*Number*	*Per Cent*	*Number*	*Number*	*Per Cent*
Pyrethrum extract	42	475	82	41	343	76	120	408	41
Cottonseed oil	138	341	19	255	660	23	113	503	55
Kerosene	440	864	−2	395	739	−7	98	184	−7

as follows: Suppose that 200 worms are caught by a certain number of bands having treated and untreated halves, and that 40 of these are caught in the treated halves. Had these halves also been untreated, we would have expected that they would have captured one-half of the total number of worms, or 100. However, as these treated halves captured only 40, the number of worms repelled may be considered to be the difference between 40 and 100, or 60. The repellency is therefor 60 per cent.

The pyrethrum extract used had a pyrethrin content of 2.5 per cent by weight, or 2.0 gm. per 100 cc. The kerosene used was of undetermined specifications. Crude cottonseed oil was used.

RESULTS.—When bands were impregnated with undiluted pyrethrum extract, cottonseed oil, or kerosene (Table 1), only pyrethrum extract was highly repellent. Cottonseed oil showed some repellency, but kerosene appeared to be neither repellent nor attractive. Pyrethrum extract at 100 per cent showed more repellency than cottonseed oil over periods of 30 and 60 days of exposure, but the latter was more repellent than the former over an exposure period of 90 days. Repellency of pyrethrum extract decreased throughout the 90-day period, while that of cottonseed oil increased. Pyrethrum extract killed about 50 per cent of the established larvae, but kerosene killed practically none.

Further tests (Table 2) confirmed the repellent effect of pyrethrum extract used at full strength, and showed it to have considerable repellency when used in combination with kerosene. The repellency decreased with reduced percentages of pyrethrum extract, and that of any concentration fell off between 30 and 90 days. A dilution of 5 per cent of pyrethrum extract and 5 per cent of kerosene gave 80 per cent of repellency at 30 days, but decreased to only 20 per cent at 90 days. Very little repellency was shown by a dilution of 2 per cent of pyrethrum extract and 5 per cent of kerosene.

To test pyrethrum extract and cottonseed oil on a more practicable basis, experiments were made with the two materials at greatly reduced concentrations, and tests were also made with combinations of the diluted materials. These are summarized in table 3. Pyrethrum extract

Table 2.—Repellency of pyrethrum extract and kerosene to mature codling moth larvae. Yakima Wash.

Material		Larvae Caught in Bands and Repellency of Materials After Exposure of—								
		30 Days			60 Days			90 Days		
		Larvae in Bands		Repellency	Larvae in Bands		Repellency	Larvae in Bands		Repellency
Pyrethrum Extract	Kerosene	Treated	Total		Treated	Total		Treated	Total	
Per Cent	*Per Cent*	*Number*	*Number*	*Per Cent*	*Number*	*Number*	*Per Cent*	*Number*	*Number*	*Per Cent*
[1]100	—	49	608	84	46	449	80	120	447	46
50	50	75	457	67	84	273	39	148	369	20
25	75	73	450	68	251	650	23	271	634	15
12.5	87.5	237	694	32	290	655	11	250	560	11
6.25	93.75	95	275	31	110	290	24	220	500	12
3.0	97.0	134	329	19	30	100	40	135	300	10
1.0	99.0	173	396	13	391	793	1.4	451	905	0.3
—	100.0	400	864	−2	395	739	−7	98	184	−7
2	[2]5	31	67	7.5	62	137	9.5	81	168	3.6
5	[2]5	9	89	80	33	239	72	65	162	20

[1] Five tests.
[2] Blood-albumin emulsifier, 2 ounces per 100 gallons.

Table 3.—Repellency of dilutions of pyrethrum extract and cottonseed oil to mature codling moth larvae. Yakima, Wash.

Material		Larvae Caught in Bands and Repellency of Materials After Exposure of—								
		30 Days			60 Days			90 Days		
		Larvae in Bands			Larvae in Bands			Larvae in Bands		
Pyrethrum Extract	Cottonseed Oil	Treated	Total	Repellency	Treated	Total	Repellency	Treated	Total	Repellency
Per Cent	*Per Cent*	*Number*	*Number*	*Per Cent*	*Number*	*Number*	*Per Cent*	*Number*	*Number*	*Per Cent*
[1]2	0	35	76	7.9	239	515	7.2	395	878	10.0
[2]5	0	47	145	35.2	177	518	32.0	239	576	17.0
0	[2]5	168	357	5.9	126	407	39.0	187	592	37.0
0	[2]10	18	38	5.5	194	523	26.0	363	938	23.0
2	[2]5	24	98	51.0	35	160	56.3	34	231	71.0
[3]2	10	18	163	78.0	93	461	60.0	166	845	61.0
2	[2]10	4	38	79.0	17	118	71.0	21	142	70.0
[3]2	20	19	129	71.0	36	271	73.0	86	684	75.0
5	[2]5	0	92	100.0	3	176	97.0	18	436	92.0
[3]5	10	7	153	91.0	88	611	71.0	64	425	70.0
5	[2]10	0	49	100.0	0	116	100.0	3	370	98.5
[3]5	20	17	319	89.0	82	589	73.0	144	1,014	72.0

[1] Emulsified with 1 per cent of sodium oleate.
[2] Emulsified with 2 per cent of sodium oleate.
[3] Emulsified with blood albumin, 2 ounces per 100 gallons.

alone at 2 per cent showed very little repellency and was only moderately more repellent when used alone at 5 per cent. Cottonseed oil alone, at either 5 or 10 per cent, had little repellent value, although the repellency appeared to increase slightly during the 60- and 90-day periods of exposure, as was the case with undiluted oil. The addition of 5, 10, or 20 per cent of cottonseed oil to 2 per cent of pyrethrum extract increased the repellency appreciably, and the addition of the same amounts of cottonseed oil to 5 per cent of pyrethrum extract increased the repellency still further. Increasing the amount of cottonseed oil from 10 to 20 per cent did not in general increase the repellency. Somewhat better results were obtained when blood albumin, rather than sodium oleate, was used as the emulsifier. The most satisfactory results were obtained with combinations of 5 per cent of pyrethrum extract and 5 or 10 per cent of cottonseed oil.

Tests with a dilution of 1 per cent of 4,6-dinitro-o-cresol indicated that this material may be slightly attractive to mature codling moth larvae over a 30-day period of exposure and that it counteracts the repellent effect of 10 or 20 per cent of cottonseed oil when combined with that material. However, the same material and combinations showed slight repellency at exposures of 60 and 90 days. Nicotine sulfate solution (40 per cent nicotine), either undiluted or at a 1 per cent strength, showed little or no repellency, when used alone or when it was mixed with the 10 or 20 per cent of cottonseed oil.

In tests for which very few larvae were available, stove oil[2] alone at 10 per cent showed no repellency whatever and in a comparison of pyrethrum extract at 1, 2, and 5 per cent with cottonseed oil and with stove oil, both at 10 per cent, the combination of cottonseed oil and pyrethrum was superior to that of stove oil and pyrethrum, especially during the 60- and 90-day periods.

Now that a fairly promising repellent has been found, efforts are being made to develop a practical application for it. Whether a repellent applied to the trees would induce a larger proportion of the larvae to enter the bands has not been determined. It has also not been determined whether the pyrethrum-cottonseed oil combination would show continued high repellency for more than 90 days. To cover the entire period during which larvae would enter the bands, a repellent material should be effective for 130 to 140 days after its application. Two applications of the repellent about 65 to 70 days apart, however, might prove more effective and economical.

SUMMARY.—Tests have been carried on since 1936 to find a material repellent to mature codling moth larvae as they search for cocooning quarters. Bands of corrugated paper, impregnated with the test materials, were placed half way around the trunks of apple trees, and the repellent

[2] Distillation range, middle 80 per cent between 194° and 259°C., U. R., 75 (California method); Saybolt viscosity at 100°F., 32 seconds.

effect was determined by comparing the numbers of larvae captured in the treated half bands with those in the untreated halves at the end of 30, 60, and 90 days.

Of some 250 formulas tested, the best results were obtained with combinations of 5 per cent pyrethrum extract with either 5 or 10 per cent of cottonseed oil, emulsified with blood albumin. Undiluted pyrethrum extract was highly repellent but the repellent effect decreased with long periods of exposure. Cottonseed oil undiluted was considerably less repellent, although its repellency increased with longer exposure. Kerosene alone was neither repellent nor attractive and when combined with pyrethrum extract reduced the repellency of that material.

Dilutions of 1 per cent of dinitro-o-cresol, alone and in combination with 10 or 20 per cent of cottonseed oil, appeared slightly attractive during a 30-day exposure, but showed slight repellency over longer periods. Nicotine sulfate (40 per cent) at 1 per cent dilution was at most slightly repellent, alone or mixed with cottonseed oil. Stove oil, used at 10 per cent, was not repellent.—6-16-44.

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A Dust Experiment for Codling Moth Control in a Heavy Infestation[1]

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Wartime shortages of labor and spraying machinery have aroused and renewed interest in dusting for control of the codling moth, *Carpocapsa pomonella* (L.), in New York. Harman (1944) has reported on such a dusting experiment. Satisfactory commercial control has been obtained with careful timing and application of improved dust mixtures in areas of light to moderate infestations. In Wayne County, where infestations rarely exceed 35 per cent entrances, dusting experiments have been made annually on a Cornell University project since the season of 1937. The present experiment was designed to test the methods and mixtures developed by Dr. Glenn E. Carman[2] and the writer, which had given good control in Wayne County, in a region 100 miles farther west, where the codling moth is generally a more serious pest. The trees in the test orchard, near Kuckville, Orleans County, were McIntosh, from which 10 to 12 bushels per tree were harvested.

While the principal purpose of the test was to find out what control would result with a dusting program in a heavy infestation, at the same time an attempt was made to determine the advisability of increasing the *Black Leaf 155* supplement in a lead arsenate dust from 10 to 20 per cent. The third material tested was a proprietary dust.

PROCEDURE AND EXPERIMENTAL DESIGN.—The dusts were applied with a Niagara Cyclone model orchard duster powered with a tractor take-off. The trees were dusted from opposite sides at each application. Applications were made at night or in the early morning when atmospheric conditions were as favorable as possible. A slight amount of dew was present at the time of the sixth application, and at the tenth application the foliage and fruit were wet with dew. In all other cases no detectable amount of dew was present.

An average of 2 lbs., 6 oz. of dust per tree per application was applied regardless of tree size. Considerable success was attained in applying the different materials at the same rate per tree, the average greatest difference in rate for a single application being $2\frac{3}{4}$ oz. per tree.

In the test orchard, trees were spaced at 25 foot intervals down the row, with a

[1] This is a report on a portion of the investigations carried on by the Department of Entomology at Cornell University under a fellowship sponsored by the Tobacco By-Products and Chemical Corporation, Louisville, Kentucky, and is part of a coordinated program on fruit insect control between the Department of Entomology at the Cornell University Agricultural Experiment Station and the Division of Entomology, N. Y. State Agricultural Experiment Station, Geneva, N. Y.

[2] Carman, Glenn E. 1942. Studies on the Control of Codling Moth, *Carpocapsa pomonella* Linn. with Dusts. Unpublished thesis, Cornell University Library, Ithaca, N. Y.

distance of 40 feet between rows. The three treatments described below were applied to three adjoining latin squares. Two of the squares were composed of single tree replicates. The third square was made up of plots consisting of three consecutive trees each. The counts in the latter case were taken from the center tree of each plot.

MATERIALS TESTED.—

Treatment 1. 20 per cent lead arsenate
20 per cent *Black Leaf 155* (equivalent to 2.8 per cent nicotine for the mixture)
60 per cent *Micronized Dusting Sulfur*

Treatment 2. A proprietary mixture chosen because it was of a type being sold to local growers as a codling moth dust. This dust was made up by request to contain the same proportions of lead arsenate and *Black Leaf 155* as treatment 1. The carrier used was not disclosed by the manufacturer. Preparations of this type sold to growers generally contained not more than 10 per cent *Black Leaf 155* in addition to 20 per cent lead arsenate.

Treatment 3. 20 per cent lead arsenate
10 per cent *Black Leaf 155* (equivalent to 1.4 per cent nicotine for the mixture)
70 per cent *Micronized Dusting Sulfur*

SCHEDULE OF APPLICATIONS.—The test began with the first cover application, the cooperating grower having applied a uniform calyx spray of lead arsenate. Seven cover dusts were applied for first brood larvae. These were applied on June 22, 28, July 3, 7, 13, 18, and 24. Three dusts were applied against second brood larvae. These were applied on August 10, 20, and 28. The third and tenth cover dusts were applied during periods when codling moth activity was low and consisted of lead arsenate 20 per cent, *Micronized Dusting Sulfur* 80 per cent for all plots. This unsupplemented dust was used in this manner in order to reduce the cost of the program. All other cover dusts consisted of the listed treatments.

SECURING AND CALCULATING THE DATA.—From time to time during the season, the drop apples were examined and injuries recorded. After harvest the remainder of the dropped apples was examined. Where the number of these was excessive, a sample of 300 apples was taken from the pile for examination and the number remaining determined. It was necessary to make extensive counts of the dropped apples, since with the McIntosh variety most of the entered apples fall to the ground. At harvest a sample of 300 fruit was examined from each count tree. To obtain this sample, an equal number of apples was taken from each harvest crate. In making both drop and harvest counts, each apple was placed in one of five classifications,—uninjured, entered (including entered apples with stings), one sting, two stings, and over two stings. For the purposes of this paper the data for unentered, stung fruit has been grouped into one classification.

An estimate of the total number of harvest fruit was made by taking an equal number of apples from each harvest crate in sufficient quantity to fill two crates of similar size. The number of harvest fruit could then be estimated by multiplying the average number of apples per crate by the total number of harvest crates.

From the above data it was possible to figure the per cent of entered and stung fruit for the total crop of each count tree. The percentage figures for entrances were transformed to their corresponding angles and treated by an analysis of variance as given by Paterson (1939) for replicated latin squares. The analysis of variance for per cent stung fruit was carried out in a similar manner except that the transformation was not made.

At harvest, for residue analyses, samples of fruit were taken in a representative manner from the east and west halves of one tree representing each treatment. A third sample was taken from the west half of each of the same trees and run through an apple brusher. It was not anticipated that any great differences in residue would be found among the treatments, since each treatment contained the same amount of lead arsenate. Rather, a number of samples was taken in order to determine whether any of them would exceed the tolerance for arsenic.

RESULTS.—The average per cent of entered and stung fruit for the different treatments is given in table 1.

The differences between the treatments in control of both entrances and stings were such as to be not mathematically significant when a generalized error is used. Treatment 1 was best in control of entrances in 11 out of the possible 18 groupings into columns and rows, the other materials varying considerably more in their relative placement.

Table 2 presents analyses of residues of As_2O_3 present on the fruit at harvest.

Table 1.—Codling moth injury to fruit from dusted trees.

Treatment[1]	Entered[2] Per Cent	Stung Per Cent
1. 20% Lead arsenate 20% *Black Leaf 155* 60% *Micronized Dusting Sulfur*	15.0	30.8
2. 20% Lead arsenate 20% *Black Leaf 155* Proprietary diluent not known	20.6	31.5
3. 20% Lead arsenate 10% *Black Leaf 155* 70% *Micronized Dusting Sulfur*	19.3	30.0

Differences not mathematically significant.

[1] Trees received a total of 8 applications of these materials. All trees received 2 applications of 20% lead arsenate, 80% *Micronized Dusting Sulfur*.

[2] Includes apples entered and also stung.

Table 2.—As_2O_3 residue analyses of apples from dusted plots.[1]

Treatment No.	Grains As_2O_3 per Pound of Apples		
	Before brushing		After brushing[2]
	East Half	West Half	West Half
1.	0.017	0.020	0.013
2.	0.018	0.025	0.016
3.	0.017	0.016	0.013

[1] Residue analyses were made by Dr. George W. Pearce, Chemistry Division, N. Y. State Agricultural Experiment Station, Geneva, N. Y.

[2] Samples were run through an *Improved Prescott Fruit Cleaner*.

Although a total of 10 dusts were applied, it will be seen that in no case did the residue exceed the domestic tolerance for As_2O_3 (0.025 grains per pound). In one case this tolerance was equalled. The data from the brushed samples would indicate that this treatment would be effective in reducing dusted apples which received a lead arsenate calyx spray and are found to be slightly in excess of the tolerance to within its limits. In connection with this low level of residue, the absence of dew in eight out of ten applications should be noted.

Discussion.—From table 1 it will be seen that considerable codling moth injury resulted with all treatments under the conditions of the test. A single tree located in a row between the second and third squares was undusted except at the time of the third and tenth applications when it received a 20 per cent lead arsenate dust. The data from this tree, intended to give some idea of the level of infestation, was 58.3 per cent entered and 17.3 per cent stung. It would appear that a dusting program such as indicated will afford a considerable measure of control. The dusting programs represent an investment for materials of approximately 29 cents per bushel of harvested fruit for schedules 1 and 2, and 21 cents per bushel for schedule 3. Applying an excellent spray program for codling moth control, consisting of from 2 to 4 quarts of summer oil, 3 to 5 pounds of lead arsenate, and 2 pounds of *Black Leaf 155* per hundred gallons, with appropriate fungicide and arsenical corrective, the cooperating grower spent approximately 15 cents per bushel for materials. Spraying from the tank, he took a little more than twice as much time per tree in making six spray applications as was necessary for 10 dust applications. Counts were taken from three sprayed trees adjoining the dust plots. These indicated codling moth injury of 9.2 per cent entrances and 35.0 per cent stings. As in the case of the check tree data, this information is not strictly comparable with the data from the dusted trees. It is presented simply as additional information on the degree of infestation in the test orchard.

To determine whether it is profitable to increase the concentration of *Black Leaf 155* from 10 per cent to 20 per cent, the increased returns in clean fruit should be taken into account. The results of this test allow only speculation in this regard, since differences were not shown to be mathematically significant. Only treatments 1 and 3 are comparable on this score, since their composition is known to be alike in other respects. If we assume that repeated tests would indicate that the higher concentration of *Black Leaf 155* protects about 4 per cent more fruit from entries, as in this test, then under like conditions of infestation, size of crop, and rate of application, one more bushel of clean fruit would represent an increase in investment for materials of approximately $1.70. Whether this would be worth while would depend upon market conditions.

If the difference in control of entrances between treatments 1 and 2 should prove by repeated experimentation to be real,

it could only be ascribed to a difference in the diluent used.

A rough attempt was made to measure by analysis of covariance, removing effects of blocks (columns) and treatments only, the correlation, if any, between the size of crop and the per cent of entrances. The coefficient of regression resulting from this analysis was positive but not mathematically significant. The relationship between crop size and per cent of entrances, when variations in crop size are due to differences in set, has been found by various workers to be generally negative. However, if variations in crop size are due mainly to variations in size of tree, then where an equal dosage per tree is applied, as in this experiment, a positive relationship might exist, since the smaller trees received more dust per unit volume. If this relationship holds true, then an increase in overall rate of application over that used may be expected to result in an improvement in control.

SUMMARY.—Three dusts consisting respectively of (1) 20 per cent lead arsenate, 20 per cent *Black Leaf 155*, and 60 per cent *Micronized Dusting Sulfur*, (2) the same proportions of lead arsenate and *Black Leaf 155* with a proprietary diluent, and (3) 20 per cent lead arsenate, 10 per cent *Black Leaf 155*, and 70 per cent *Micronized Dusting Sulfur* were applied in 8 cover dusts to three latin squares laid out in an orchard relatively heavily infested with the codling moth, and located in Orleans Co., N. Y. In addition, all plots received two cover dusts of 20 per cent lead arsenate and 80 per cent *Micronized Dusting Sulfur*. Dusts were applied from both sides at the average rate of 2 lb., 6 oz. per tree per application.

Codling moth injury in the dusted plots ranged from 15 per cent to 20 per cent entrances with approximately 30 per cent stung fruit. In no case were differences mathematically significant. The degree of control obtained in this test would indicate that a dusting program of this type should be resorted to in a similar infestation only if, because of labor shortages or other conditions, it is not possible to spray. In the latter instance, a considerable reduction in codling moth damage may be expected from similar dusting programs. Indications are that an increase in the amount of dust per tree over that used in this test would result in an improvement in the control of entrances. None of the samples taken at harvest exceeded the domestic tolerance for residue of As_2O_3. This is thought to be largely attributable to the infrequency of dew when applications were made.—5-3-44.

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The Mexican fruitfly quarantine regulations as revised effective July 3, 1944, provide for a harvest season for grapefruit, sweet limes, and "sour" and "bittersweet" oranges from September 1 through June 15 of each year for all the regulated area, and for a year-round harvest season for commercial varieties of sweet oranges, kumquats, tangelos, and all varieties of the mandarin group of oranges. Under former regulations the period during which the host fruits were permitted to be harvested for shipment interstate closed on April 30 except that the grapefruit harvest in three counties closed on the last day of February.

Coastal Chemical Company, Inc., of Harlingen Texas, manufacturers and distributors of agricultural insecticides and fungicides, has been taken over by Niagara Sprayer and Chemical Co., Inc., Middleport, New York.

Niagara Sprayer and Chemical Co., Inc., a subsidiary of Food Machinery Corporation, San Jose, California, will have the operation and management of the Coastal Chemical Company plant and business and will merge these operations with their own.

Extensions and modernization of the present Coastal Chemical Company plant are in the process of being made by the new management.

Effect of Scraping and Banding Trees Upon the Numbers of Transforming and Hibernating Codling Moth Larvae

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Bands applied to scraped trees in wormy orchards capture large numbers of codling moth larvae, but estimates as to the percentage of larvae they catch vary widely, and more or less according to the basis on which they are made. Most estimates are based on a comparison of the number of larvae captured in bands with the number found to have left the fruit or with the number found in hibernation. Estimates of the percentage of band-caught larvae based on the number found to have left the fruit do not take into account mortality of larvae in their search for cocooning quarters, and thus the value of scraping and banding from the standpoint of their effect on the developing larval population is underestimated. On the other hand, estimates of the percentage of band-caught larvae based on the number found in hibernation fail to take into account the emergence of moths that develop from transforming larvae during the season, and thus the value of scraping and banding from the standpoint of their effect on the developing larval population is overestimated.

An estimate of the percentage of larvae caught in 2-inch, untreated, corrugated-paper bands applied to the trunks of well-scraped trees was made in orchards near Troy, Kans., in 1936 and 1937. Record was made of the larvae in the bands at 10-day intervals from the time larvae began leaving the fruit until harvest, and once more after harvest. On the basis of the number of larvae found to have left the fruit, the bands captured 15 per cent of the larvae on 72 21-year-old Jonathan trees in 1936 and 20 per cent of the larvae on 40 20-year-old Jonathan trees in 1937. Other estimates of the percentage of codling moth larvae caught in the trunk bands range from 10 per cent on the total number that entered fruit (Steiner & Ackerman 1936) to from 15 per cent (Harman 1933), 28 per cent (Cutright 1937), and 43 per cent (Yothers & Carlson 1941b), to about 50 per cent of the total number of larvae found to have left the fruit (Newcomer et al. 1933). Van Leeuwen (1929) reported that a burlap band applied to the trunk of a thoroughly scraped tree captured 20 per cent of the many larvae placed on the tree. Additional estimates of the percentage catch of codling moth larvae in bands, based on the number of larvae found in hibernation after harvest, range from 28 to 73 per cent (Woodside 1938), 69 to 83 per cent (Woodside 1941), and 73 to 76 per cent (Farrar & Flint 1933) to 96 to 97 per cent (Worthley 1932, 1934). Worthley (1932, 1934) reported moderately lower percentage catches when examinations of trees for hibernating larvae were supplemented with examinations for pupal skins at intervals during the summer and fall. A few investigators—Steiner & Ackerman (1936), Yothers (1940), and Yothers & Carlson (1941a)—have reported on the effect of scraping and banding on codling moth control.

The variation in the foregoing estimates of the percentage catch of codling moth larvae in bands is too great to suggest that it can be due entirely to sectional differences. It must be due, in part at least, to the methods of estimation used. Weaknesses in the methods of estimation used most commonly have been mentioned. An experiment was planned and carried out in orchards near Troy, Kans., during the growing seasons of 1938, 1939, and 1940 to take these weaknesses into consideration by using the number of larvae that develop as the basis for estimating the percentage catch of larvae in bands, rather than the number of larvae leaving the fruit or the number found in hibernation after harvest. For the purpose of this investigation, the number of larvae that developed included (1) larvae that developed into moths during the current season, (2) live larvae in hibernation shortly after harvest, and (3) larvae captured in bands. Of course, larvae in hibernation after harvest may not complete development into moths, but winter mortality of codling moth larvae has been studied by other workers and is not considered an essential part of this

[1] Kenneth Lamansky and the late E. W. Cook assisted in the work.

investigation. This experiment was so designed as to furnish information on the effect of scraping trees, and of scraping and banding trees, on the developing larval population.

PROCEDURE.—Each season 15 trees in as compact a block as possible were selected for uniformity as to size of tree and crop and were divided at random into 3 series of 5 single-tree replicates. One series was scraped and not banded, one was scraped and banded, and one served as an unscraped, unbanded check. In 1938 and 1939 the trees were located in a block of 18- or 19-year-old Jonathan, and in 1940 in a block of 23-year-old Delicious. They were sprayed by the grower according to the program in use in the remainder of the orchard. The trees in the 2 treated series were scraped thoroughly early in the spring and the ground beneath all trees was cleaned thoroughly of grass, weeds, and trash, to force as many larvae as possible to cocoon on the trees, and to facilitate examinations for pupal skins and hibernating larvae. Bands, where used, were of single-faced, untreated, corrugated paper 2 inches wide and were applied to the tree trunks.

The total number of mature larvae that left the fruit was determined by examination of all dropped and harvested fruit for exit holes. The number that developed into moths was determined by a thorough examination of all trees, and the ground beneath them, for pupal skins, three times each week until harvest and once each week thereafter to the end of the season. The number that entered hibernation was determined by a thorough examination of all trees and the ground beneath them for live, hibernating larvae shortly after harvest was completed. Bands were removed, examined for captured larvae, and replaced at 10-day intervals during the season until harvest and once more following harvest. Captured larvae were destroyed.

RESULTS.—A comparison of the percentages of codling moth larvae caught in bands in this experiment when different bases for their estimation were used is given in table 1. The bands captured 331 larvae in 1938, 673 larvae in 1939, 761 larvae in 1940, and a total of 1765 larvae during the 3 years.

The data in table 1 show that the percentage catch varies widely according to the basis on which it is estimated. The lowest estimate is that based on the number of larvae that left fruit and the highest estimate is that based on the number captured in bands plus the number found in hibernation after harvest. The estimate based on the number of larvae that developed into moths or entered hibernation is intermediate between the others and is believed to indicate more accurately than the others the extent to which bands on scraped trees reduce the number of codling moth larvae.

Table 1.—Comparison of the percentages of codling moth larvae caught in bands when different bases for their estimation were used, Troy, Kans., 1938–40.

Basis of Estimation	Catch			
	1938	1939	1940	All Years
	Per Cent	*Per Cent*	*Per Cent*	*Per Cent*
Larvae that left fruit	29.3	36.0	24.9	29.1
Larvae in hibernation[1]	92.2	90.7	80.5	86.3
Larvae that developed	74.2	74.7	60.3	67.7

[1] Plus larvae captured in bands.

The effect of scraping and of scraping and banding trees upon the numbers of transforming and hibernating codling moth larvae is shown in table 2, together with the differences between treatments, calculated by analyses of variance, necessary for two levels of significance.

Only 29 per cent of the larvae leaving the fruit in 1938, 26.4 per cent in 1939, 41.2 per cent in 1940, and 35.2 per cent in all years combined were subsequently accounted for as emerged moths or hibernating larvae on the check trees. These data indicate that, under Missouri River Valley conditions at least, there is a very heavy natural mortality of the codling moth between the time that it leaves the fruit as a mature larva and the time that it emerges as a moth or enters hibernation.

Scraping, and to a greater extent scraping and banding, effected material reductions in the percentage of larvae that developed into moths and also in the percentage entering hibernation. Using the combined data for all years, 35.2 per cent of the larvae that left the fruit on check trees developed into moths or entered hibernation, while 20.7 per cent did so on the scraped trees, and only 13.9

Table 2.—Effect of scraping and of scraping and banding trees upon the numbers of transforming and hibernating codling moth larvae, Troy, Kans., 1938–40.

Treatment of Trees	Total Larvae Leaving Fruit	Larvae Leaving Fruit That—		Reduction in—	
		Developed into Moths	Entered Hibernation	Moth Emergence	Larvae Entering Hibernation
	Number	*Per Cent*	*Per Cent*	*Per Cent*	*Per Cent*
			1938		
None	1,019	16.8	12.2		
Scraped	1,141	13.8	4.4	17.5	64.0
Scraped and banded	1,129	7.7	2.5	54.0	79.6
Difference required for significance: P=.05		5.0	3.9		
P=.01		7.0	5.4		
			1939		
None	1,599	13.4	13.0		
Scraped	1,764	13.8	3.1	0.0	76.0
Scraped and banded	1,870	8.5	3.7	36.5	71.6
Difference required for significance: P=.05		[1]	5.7		
P=.01		[1]	8.0		
			1940		
None	3,365	19.2	22.0		
Scraped	2,658	14.1	10.2	26.4	53.9
Scraped and banded	3,057	10.3	6.0	46.1	72.7
Difference required for significance: P=.05		4.9	3.0		
P=.01		6.9	4.2		
			Total (all years)		
None	5,983	17.2	18.0		
Scraped	5,563	14.0	6.7	18.6	62.8
Scraped and banded	6,056	9.3	4.6	45.9	74.4
Difference required for significance: P=.05		2.6	2.5		
P=.01		3.5	3.3		

[1] The *F* test indicated treatment differences not quite significant.

per cent did so on the scraped and banded trees.

The reduction in the number of transforming larvae that developed into moths on the scraped, unbanded trees as compared with the check trees was not significant in any one season but the reduction over the 3-year period was significant. This indicates that one may expect to obtain a reduction in moth emergence merely by scraping his trees.

Reductions in the number of larvae that entered hibernation on the scraped, unbanded trees as compared with the check trees were all highly significant, so it may be stated that scraping without banding may be expected to effect a greater reduction in the nontransforming than in the transforming larval population and to effect a very material reduction in the number of larvae that enter hibernation.

Reductions in the number of transforming larvae that developed into moths on the scraped and banded trees as compared with the checks were all highly significant except in 1939, when the reduction was not quite significant. This was due in part to the fact that the trees used suffered a heavy attack of twig blight and many transforming larvae found suitable cocooning quarters in the dried leaves on the blighted twigs. The data indicate that the use of bands on scraped trees may be expected to effect a material reduction in the number of transforming larvae that develop into moths.

Reductions in the number of larvae that entered hibernation on the scraped and banded trees as compared with the number on the scraped, unbanded ones were small and not significant, but as compared with the checks these reductions were all highly significant and in-

Table 3.—Type of location and section of tree in which pupal skins of codling moths of the transforming broods were found on check, scraped, and scraped and banded trees, Troy, Kans., 1938.

Treatment	Location	Number of Pupal Skins and Total Percentage Found: On Exposed Roots and in Cracks	Under Bark	In Crotches and Forks of Limbs	In Pruning or Other Scars	In Cracks and Splintered Limbs	In Other Locations on Trees[1]	Total	Per Cent
Check	On ground	14						14	8.2
	On tree								
	Lower third		59	37	20	3	1	120	70.6
	Middle third		3	6	13	3	2	27	15.9
	Upper third		0	0	4	5	0	9	5.3
	Total	14	62	43	37	11	3	170	
	Per cent	8.2	36.5	25.3	21.8	6.5	1.7		
Scraped	On ground	28						28	18.4
	On tree								
	Lower third		9	19	41	6	6	81	53.3
	Middle third		3	3	23	5	1	35	23.0
	Upper third		0	0	5	2	1	8	5.3
	Total	28	12	22	69	13	8	152	
	Per cent	18.4	7.9	14.5	45.4	8.6	5.3		
Scraped and banded	On ground	8						8	9.4
	On tree								
	Lower third		2	13	21	3	7	46	54.1
	Middle third		0	5	13	5	0	23	27.1
	Upper third		0	1	5	2	0	8	9.4
	Total	8	2	19	39	10	7	85	
	Per cent	9.4	2.4	22.4	45.9	11.8	8.2		

[1] Other locations include bird droppings on limbs, between limbs and leaves, fruit on trees, and markers on trees.

dicative of the value of scraping and banding to reduce the population of hibernating codling moth larvae.

DISTRIBUTION OF COCOONING QUARTERS OF TRANSFORMING LARVAE.—During the 1938 season, record was maintained of the type of location and section of the tree in which pupal skins of the transforming broods of the codling moth were found. The data are given in table 3.

The data indicate that transforming larvae prefer to cocoon under bark, in crotches, in forks of limbs, or in pruning or other scars, and that when bark is removed by scraping many still cocoon in the other situations mentioned. It is to be noted that quite a few transforming larvae cocooned off the trees, especially when bark or bands were not present to provide cocooning quarters on the trees. Many more larvae cocooned in the lower third of the trees, as would be expected, than in the upper two-thirds. Scraping, or scraping and banding, had no noticeable effect on the number of larvae that cocooned in the upper two-thirds of the tree but did aid materially in reducing the number that cocooned in the lower third.

No record was kept of the location of hibernating larvae, since a number of workers have reported on that phase of the codling moth problem.

SUMMARY.—The effect of scraping and scraping and banding trees on the ability of transforming codling moth larvae to develop into moths and of nontransforming larvae to enter hibernation was studied in replicated plots in commercial orchards during the seasons of 1938, 1939, and 1940. It was shown that estimates of the percentage catch of larvae in bands varied widely according to the basis on which they were made. The lowest estimate was that based on the number of larvae that leave the fruit, the intermediate estimate was that based on the number of developing larvae, and the highest

estimate was that based on the number of larvae caught in the bands plus the number found in hibernation after harvest. The estimate based on the number of larvae developing is believed to indicate more accurately than the others the actual extent of the effect of banding on codling moth populations.

It was found that, under Missouri River Valley conditions, there is a very heavy mortality of the codling moth between the time when it leaves the fruit as a mature larva and the time when it emerges as a moth or enters hibernation. Nearly as many larvae transformed and developed into moths as entered hibernation on the check trees, more did so on the trees that were scraped, and still more did so, not counting those in the bands, on the trees that were scraped and banded. Scraping and, to a greater extent, scraping and banding effected material reductions both in the number of larvae that developed into moths and in the number that entered hibernation. Of all larvae that left the fruit during the 3 years, 35.2 per cent of those on the check trees developed into moths or entered hibernation, while 20.7 per cent did so on the scraped trees, and only 13.9 per cent on the trees that were scraped and banded. The effect of banding on moth emergence and larval hibernation was much greater than that indicated by the percentage of the larvae that left the fruit that was caught in bands. Transforming larvae were found to cocoon in greatest numbers in the lower third of the trees and under bark, in crotches or forks of limbs, or in pruning or other scars. —4-24-44.

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Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity as Fumigants[1]

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The possibility of using certain pyridine derivatives as fumigants was suggested to the authors by the work of Jewson & Tattersfield (1922), who used pyridine as a fumigant for certain mites in fungus cultures. With this work in mind, pyridine and 12 of its derivatives were tested as fumigants against the common red spider, *Tetranychus telarius* (L.). These preliminary tests indicated definite relationships between chemical structure and toxicity. A more extensive experiment using the confused flour beetle, *Tribolium confusum* Duv., as a test insect was then planned. During the time required for the development of a culture of flour beetles, 28 compounds were tested as fumigants against the large milkweed bug, *Oncopeltus fasciatus* (Dall.), quantities of which were readily available. These tests were discontinued as soon as the flour beetles were ready to use. The results reported in this paper include the preliminary tests against red-spiders and milkweed bugs as well as the final tests against flour beetles.

The pyridine derivatives tested were furnished by the Reilly Tar and Chemical Corporation and have been discussed in detail in an earlier paper (King & Frear, 1943). All the materials tested are listed in table 2.

Procedures.—*Greenhouse red spiders*—Two 6-inch provision jars were used for each fumigation chamber, one jar being inverted over the other and the two sealed together with cellulose tape. The total volume of such a chamber was 4800 ml. Potted string bean plants infested with mites were placed in the chambers, the required amount of fumigant introduced and the chamber sealed for five hours. At the end of this interval the chambers were opened and the plants were exposed to fresh air for 24 hours before making counts. After some preliminary trials a concentration of 3.0 milligrams of toxicant per liter of space was chosen. The temperature was maintained at 25° C. and duplicate tests were made of all materials. The average infestation was 85 mites per plant.

Milkweed bugs.—Four-liter erlenmeyer flasks (actual capacity 4500 ml.) with paraffined rubber stoppers were used as fumigation chambers. The required amount of fumigant, measured with a pipette and absorbed on a small piece of cellucotton was dropped into the flask. The flask was stoppered for an hour to allow the fumigant to volatilize. Twenty milkweed bugs (10 of each sex) were then introduced into the chamber and exposed to the fumigant for five hours. During the series of fumigations the temperature ranged from 27° to 30° C. After exposure the bugs were removed to a clean recovery cage containing fresh food and water. Mortality records were taken after 48 hours. Since the numerous controls showed no mortality the per cent kill in the test groups was reported directly as such.

Each material was tested first at a concentration of 2.0 milligrams per liter. Materials giving a high kill at this concentration were retested at lower concentrations, while the less effective compounds were retested at higher levels. Each compound was tested only once at any one concentration. For 24 of the materials, the results were such that they gave reasonably straight lines when plotted on logarithmic-probability paper. Median lethal concentrations were estimated for these compounds.

Flour beetles.—The technique was similar to that described above for the fumigation of milkweed bugs. The flour beetles were confined in small cylindrical cages of fine wire screen. During the five-hour exposure period these cages were suspended from the rubber stoppers of the chambers. The beetles were placed in clean whole wheat flour for the recovery period and

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[2] Former Reilly Research Fellow and Professor of Agricultural and Biological Chemistry, respectively. This work was partially supported by a grant from the Reilly Tar and Chemical Corporation, of Indianapolis, Indiana, and was carried out in the Department of Agricultural and Biological Chemistry in cooperation with the Department of Zoology and Entomology, and at the suggestion and with the cooperation of the Research Division of the Reilly Tar and Chemical Corporation.

mortality counts were made one week after exposure. Fifty beetles, selected at random, were used for each test. Each material was tested at a concentration of 5.0 milligrams per liter and then at higher or lower levels, as indicated by the results of the first test. An attempt was

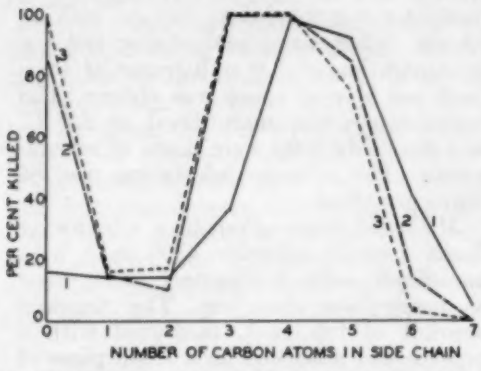

FIG. 1. (1) Effect of length of side chain on toxicity of 2-n-alkyl-pyridine fumigants to greenhouse red spider. Concentration 3.0 mg./l. (2)—Effect of length of side chain on toxicity of 2-n-alkyl-pyridine fumigants to milkweed bugs. Concentration 2.0 mg./l. (3)—Effect of length of side chain on toxicity of 2-*n*-alkyl-pyridine fumigants to flour beetles. Concentration 10 mg./l.

made to cover the range of toxicity for each material. In many cases several replications were made at critical concentrations. The number of concentrations at which each material was tested and the number of beetles counted are listed in table 2. Of the 42 materials tested, median lethal concentrations were estimated for 30. At the temperatures which obtained during these tests, *i.e.* 24°–30° C., the remaining 12 materials were not toxic, at the saturation point.

DISCUSSION OF RESULTS.—In this as well as in earlier papers (1944, 1944a) emphasis was placed on a study of the relationships between length and position of the side chains and toxicity to various organisms. Figure 1 (1) illustrates the effect of the length of the side chain on the toxicity of 2-*n*-alkyl-pyridine fumigants to red spider and shows the peak of toxicity that occurs at the butyl and amyl derivatives. At the time these tests were made the complete series of 4-alkyl-pyridines was not available. 4-*n*-amyl-pyridine and 4-*n*-propyl-pyridine were available, however, and tests of these two compounds indicated that they were more toxic than the corresponding 2-alkyl derivatives. Tests of a partial series of 2-*n*-alkyl—6-methyl-pyridines indicated that the toxicity of this series was less than that of either the 2- or 4-monosubstituted series.

Figure 1 (2) illustrates the effect of length of side chain on the toxicity of 2-*n*-alkyl-pyridine fumigants to milkweed bugs. It will be observed that a peak of toxicity occurs at the propyl and butyl derivatives and that pyridine, the first member of the series, is also relatively toxic. The relative toxicity of pyridine in this experiment was much greater than in the tests using red spiders, as will be noted by comparison of curves 1 and 2.

Median lethal concentrations were established for 27 of the materials tested against milkweed bugs. Compounds which were found to have a median lethal concentration less than 0.5 mg./l. were, in order of decreasing toxicity: 4-*n*-amyl-piperidine, 2-*n*-hexyl-piperidine, quinoline and 4-*n*-amyl-pyridine. Compounds with median lethal concentrations from 0.5 to 1.9 mg./l. were 4-*n*-butyl-pyridine, 2-*n*-butyl-pyridine, 4-*n*-propyl-pyridine, 2-*n*-propyl-pyridine, 4-isopropyl-pyridine, 4-ethyl-pyridine, pyridine, 2-*n*-amyl-pyridine and 2-butyl-6-methyl-pyridine. Compounds with median lethal concentrations between 2.0 mg. and 3.0 mg./l were 2-amyl, 6-methyl-pyridine, 4-mixed-hexyl-pyridines; 2,5-dimethyl-pyridine and 2,4-dimethyl-pyridine. Compounds with median lethal concentrations ranging from 3.0 mg./l to 4.0 mg./l were 2-isopropyl-pyridine; 2-*n*-hexyl-pyridine; 2-ethyl-6-methyl-pyridine; 3-methyl-pyridine; 2-ethyl-pyridine; 2,6-dimethyl-pyridine; 2-mixed-hexyl-pyridines, 2-hexyl-6-methyl pyridine and 2-*n*-heptyl-pyridine. Compounds with median lethal concentrations between 4.0 and 10.0 mg./l. were β,β'-dichloroethyl ether; carbon disulfide; methyl acetate; 2-ethanol-pyridine and 2-*n*-octyl-pyridine. Compounds with median lethal concentrations above 10.0 mg./l were 2-methyl-pyridine; 2-(3-octyl)-pyridine; 2-(2-me-octyl)-pyridine; 2-(6-undecyl)-pyridine; 2-mixed-undecyl-pyridines; 4-*n*-hexyl-pyridine; ethylene dichloride and carbon tetrachloride.

In these tests it was again observed that the 4-substituted alkyl pyridines were more toxic than the 2-substituted compounds and that the disubstituted

compounds were less toxic than the corresponding monosubstituted compounds. It was also observed that the two piperidines tested were more toxic than the corresponding pyridines.

Table 1.—Concentrations of fumigants required to kill 50 per cent and 90 per cent of flour beetles.

Compound	Concentration (in mg./l) Required to Kill	
	50%	*90%*
Pyridine	7.15	8.35
2 methyl-pyridine	10.14	13.00
2 ethyl-pyridine	11.32	14.26
2-*n*-propyl-pyridine	4.81	5.83
2-*n*-butyl-pyridine	6.40	8.73
2-*n*-amyl-pyridine	10.81	1836.[1]
2-*n*-hexyl-pyridine	966.0[1]	15310.[1]

[1] Values above saturation point.

Figure 1 (3) shows the effect of the length of the side chain on the toxicity of the 2-*n*-alkyl-pyridine fumigants to flour beetles. As in the milkweed bug tests, a peak of toxicity occurred at the propyl and butyl derivatives and pyridine itself was relatively toxic.

For a more critical study, the data obtained in the tests of the 2-*n*-alkyl-pyridines against flour beetles were plotted and analyzed according to the method of Bliss (1935). The equation of the regression line for each compound was computed and from the regression equation the concentrations required to kill 50 per cent and 90 per cent of the beetles were calculated. These concentrations are listed in table 1, from which it may be seen that 2-*n*-propyl-pyridine was the most toxic member of the series.

The final results of the complete series of flour beetle tests are given in table 2. Median lethal concentrations were estimated for all compounds tested, except the alkyl derivatives with 6 or more carbon atoms in the side chains. These compounds were not toxic at the saturation point. Four compounds: 2-*n*-amyl-pyridine; 4-*n*-butyl-pyridine, 4-*n*-amyl-pyridine and 4-*n*-amyl-piperidine, gave erratic results which were interpreted as indicating some degree of protective stupefaction.

From the values obtained for median lethal concentrations in the series of 4-*n*-alkyl-pyridines, it is seen that the peak of toxicity in this series occurred at the propyl derivative, as in the 2-*n*-alkyl-series. It is also seen that the members of the 4-substituted series were more toxic than

Table 2.—Effects of some N-heterocyclic compounds as fumigants against the confused flour beetle.

Compound Tested	Concentrations Tested	Beetles Counted	Median Lethal Concentration mg/l
Pyridine	8	800	7.2
2-methyl-pyridine	8	1000	10.1
2-ethyl-pyridine	11	800	11.3
2-*n*-propyl-pyridine	10	800	4.8
2-isopropyl-pyridine	5	400	14.5
2-*n*-butyl-pyridine	7	800	6.4
2-*n*-amyl-pyridine	9	1200	10.8[2]
2-*n*-hexyl-pyridine	5	500	—[1]
2-mixed-hexyl-pyridine	6	450	—[1]
2-*n*-heptyl-pyridine	5	350	—[1]
2-*n*-octyl-pyridine	5	350	—[1]
2-(3-octyl)-pyridine	5	350	—[1]
2-(2-me-octyl)-pyridine	5	500	—[1]
2-(6-undecyl)-pyridine	5	350	—[1]
2-mixed-undecyl-pyridine	5	500	—[1]
3-methyl-pyridine	5	650	5.4
4-ethyl-pyridine	6	1200	7.2
4-*n*-propyl-pyridine	10	1150	3.5
4-isopropyl-pyridine	9	850	3.8
4-*n*-butyl-pyridine	10	1200	4.4[2]
4-*n*-amyl-pyridine	14	1200	8.0[2]
4-(3-pentyl)-pyridine	4	350	29.0
4-*n*-hexyl-pyridine	6	350	—[1]
4-mixed-hexyl-pyridine	5	350	—[1]
2-methyl-4-methyl-pyridine	7	750	9.0
2-methyl-5-methyl-pyridine	6	600	6.6
2-methyl-6-methyl-pyridine	10	750	6.5
2-ethyl-6-methyl-pyridine	9	1100	9.0
2-butyl-6-methyl-pyridine	6	450	4.5
2-amyl-6-methyl-pyridine	6	350	—[1]
2-hexyl-6-methyl-pyridine	6	350	—[1]
2-ethanol-pyridine	6	750	35.0
2-*n*-hexyl-piperidine	11	1000	11.0
4-*n*-amyl-piperidine	10	1500	7.6[2]
Quinoline	5	450	60.0
Carbon disulfide	5	250	55.0
Methyl acetate	5	250	82.0
Ethyl acetate	5	250	90.0
β,β'-Dichloroethyl ether	4	250	1.3
Ethylene dichloride	4	250	19.0
Carbon tetrachloride	8	400	66.0

[1] Not toxic at saturation point.
[2] Erratic results. See text.

the corresponding 2-substituted isomers. In the incomplete series of 2-*n*-alkyl,6-methyl-pyridines, the butyl compound was the most toxic, but since the propyl derivative was not available, the exact location of the peak was not determined.

The effect of hydrogenation was again illustrated by comparisons of the two piperidines with the corresponding pyridines. The median lethal concentration of 2-*n*-hexyl-piperidine was found to be 11.0 milligrams per liter, but 2-*n*-hexyl-pyridine was not toxic at the saturation point. A similar effect was not observed in a comparison of 4-*n*-amyl-piperidine and 4-*n*-amyl-pyridine.

The group of non-heterocyclic compounds listed at the end of table 2 was included for two reasons. First, it was desired to compare the alkyl pyridines with some standard fumigants such as carbon disulfide, ethylene dichloride and dichloroethyl ether. A comparison of the median lethal concentrations listed in table 2 for these compounds with those obtained for the *N*-heterocyclic compounds shows that dichloroethyl ether was more effective than any of the *N*-heterocyclic compounds but that none of the other standard fumigants tested was as toxic as the better heterocyclcic compounds.

Second, it was desired to determine median lethal concentrations for a few compounds which had been tested by other workers, as a check on the resistance of the flour beetle culture used and on the technique employed. The compounds chosen were selected from a list of materials discussed by Shepard *et al.* (1937) in reporting an experiment in which the technique was similar to that used in the work reported here. The above authors obtained as median lethal concentrations the following values: carbon disulfide, 61 mg./l.; methyl acetate, 82 mg./l.; ethyl acetate 83 mg./l; β,β'-dichloroethyl ether 1.8 mg./l.; ethylene dichloride, 37.5 mg./l.; carbon tetrachloride 185 mg./l. comparison of these values with those given in table 2 shows that the results obtained in the two experiments for carbon disulfide, methyl acetate and ethyl acetate agree closely. The difference in results obtained for ethylene dichloride was probably caused by the difference in the time between exposure and counting. Richardson & Casanges (1942) report that the effect of ethylene dichloride on flour beetles increases during the post-fumigation period to the extent that 40 to 60 per cent of the beetles are sometimes killed between the tenth and twentieth days after exposure. In the light of this work it would be expected that the mortality counts made 40 hours after exposure by Shepard and his associates would indicate a higher median lethal concentration for this compound than the counts made one week after exposure in the work reported here.

A further check on the accuracy of the results reported in this paper is afforded by the work of Richardson & Haas (1932). These authors, using an entirely different technique, found the median lethal concentration of pyridine to *Tribolium* to be approximately 7.5 milligram per liter when the exposure period was 5 hours. This is within 0.35 mg. of the value reported in the present paper.

In table 3 a summarized comparison of the 2-alkyl-pyridines and 4-alkyl-pyridines is presented. It will be noted that of the 14 pairs of data listed, the 4-substituted compound is the more toxic in eleven cases, and in the other three cases the difference is insignificant.

Table 3.—Effects of point of attachment of alkyl side chain on the toxicity of alkyl pyridine fumigants to milkweed bugs and flour beetles.

Length of Chain	Concentration Used	Per Cent Kill by 2-substituted compound	Per Cent Kill by 4-substituted compound
		Milkweed bug	
Ethyl	2 mg./l	10	100
n-propyl	2	100	100
is propyl	2	0	100
n-butyl	1	65	100
n-amyl	2	85	100
n-hexyl	2	15	40
mixed hexyl	2	0	45
		Flour beetle	
Ethyl	5	2	6
n-propyl	5	65	83
isopropyl	5	1	37
n-butyl	5	26	74
n-amyl	5	2	23
n-hexyl	5	2	0
mixed hexyl	5	6	0

Table 4 is a similar summary of paired data showing comparisons between compounds having straight side chains and those having branched side chains. Among those compounds with short side chains there is a tendency for the normal compounds to be considerably more toxic than the branched isomer. As the length of the chain increases, however, the difference between straight and branched chains decreases. In the hexyl and octyl compounds the effect seems to be reversed, the branched chain compounds being more toxic than the normal isomers.

A comparison of curves 1, 2 and 3 shows a striking similarity in the action of the 2-*n*-alkyl-pyridine fumigants on the three organisms studied, with the general range of the peak of toxicity at the compounds having 3 or 4 carbon atoms in the side chain. From a practical point of view, it is

interesting to note that for fumigation of milkweed bugs and flour beetles there is little to be gained by the addition of the side chain to the pyridine base, since pyridine itself was almost as toxic as any of the alkyl derivatives. Against red spiders, however, pyridine was relatively low in toxicity. It was expected that mites would react to fumigants in a manner different from insects, so the difference in response to pyridine was not surprising. The similarity of the curves for red spiders and for the two insects was entirely unexpected, however. It may be an indication that the alkyl pyridines tested are capable to some extent of penetrating directly through the cuticle, as Glover & Richardson (1936) have demonstrated with nicotine, pyridine and piperidine.

Table 4.—Comparison of toxicities of alkyl pyridine fumigants having straight and branched side chains.

Position and Length of Side Chain	Test Organism	Concentration Used	Per Cent Kill by	
			Normal chain compound	Branched chain compound
2-propyl	Red spider	3 mg./l.	35	5
2-propyl	Milkweed bug	2	100	0
2-propyl	Flour beetle	5	65	1
4-propyl	Milkweed bug	0.5	25	5
4-propyl	Milkweed bug	1	35	10
4-propyl	Flour beetle	2	16	0
4-propyl	Flour beetle	5	83	37
4-amyl	Flour beetle	10	52	12
2-hexyl	Red spider	3	41	30
2-hexyl	Milkweed bug	2	15	0
2-hexyl	Milkweed bug	3	20	10
2-hexyl	Flour beetle	5	2	6
2-hexyl	Flour beetle	40	12	14
4-hexyl	Milkweed bug	2	40	45
4-hexyl	Milkweed bug	3	0	75
4-hexyl	Flour beetle	10	0	17
2-octyl	Milkweed bug	5	35	70
2-octyl	Milkweed bug	10	55	35
2-octyl	Flour beetle	20	1	1

SUMMARY.—The work reported in this paper includes tests of 13 pyridine derivatives as fumigants against greenhouse red spiders, tests of 28 pyridine derivatives as fumigants against the large milkweed bug and tests of 34 materials related to pyridine as fumigants against the confused flour beetles. Emphasis was placed on a study of the relationship between toxicity and length, position and type of side chain. In all of the tests carried out it was evident that in a series of 2-*n*-alkylpyridines a pronounced peak of toxicity occurred at the propyl- or butyl-derivative. Alkyl pyridines with side chains in the 4-position were more toxic than the 2-substituted isomers and compounds with normal side chains were usually more toxic than the branched isomers. The two alkyl piperidines available were more toxic than the corresponding pyridines. The relative toxicities of the materials tested against red spiders showed a high degree of correlation with the action of the same compounds against the two species of insects studied.—4-6-44.

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Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity Against *Tribolium confusum*[1]

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During the course of a comprehensive investigation of the relationship between chemical structure and toxicity in a series of pyridine derivatives,[3] it was desired to learn something of the nature of the toxic action of these compounds against the confused flour beetle, *Tribolium confusum* Duval, and on the basis of this work to establish some relationship between chemical structure and toxicity to this insect.

Although many N-heterocyclic compounds have been tested as insecticides by various workers, among whom may be mentioned Tattersfield & Gimingham (1927), Richardson and his associates (1930, 1933, 1936), and King & Frear (1943), little is known regarding the toxicity of these materials as stomach poisons, emphasis having been placed on contact action in most cases.

The use of the confused flour beetle, *Tribolium confusum* Duval, as a test organism for toxicity experiments, although never widely practiced, is not a new method, these insects having been used by Dobroscky (1935) in measuring the relative toxicity of lead arsenate and natural cryolite and by Fassig & Campbell (1937) in studying the effectiveness of a series of homologs of paris green. A modification of the methods used by these workers was found to be satisfactory for this experiment. Although in any experiment in which the test insect is intimately in contact with the toxicant there is the possibility of action as fumigant, contact and stomach poisons, the fumigant action was reduced to a minimum in this experiment by the use of non-volatile salts.

Materials Used.—The free N-heterocyclic bases: 2-ethyl-pyridine, 4-*n*-propyl-pyridine, 4-*n*-amyl-pyridine, 2-*n*-hexyl-pyridine, 2-mixed-undecyl-pyridine, 2,6-lutidine and quinoline were furnished by the Reilly Tar and Chemical Corporation. While the commercial method of preparation of the samples used is not available for publication, a suitable laboratory synthesis of the short-chain alkyl-pyridines has been published by Tchitchibabine (1936), and Knight & Shaw (1938) have shown that higher members of the series can be prepared by a modification of Tchitchibabine's method.

Since flour beetles live and reproduce normally in a diet of whole wheat flour, it was necessary to incorporate the experimental compounds into the flour in such a way that fumigant action could be prevented, without interfering with the action of material upon the insects. With this in mind it was decided to use salts of the bases, rather than the free bases, in this experiment. The ability of pyridine to form salts with mineral acids has been known practically as long as pyridine itself, the formation of pyridine sulfate having been mentioned by Anderson (1858), the discoverer of pyridine. Some confusion regarding the exact composition of this salt has existed in the literature, however, until very recently, when the work of Peakin (1940) indicated that in the ternary system $C_5H_5N - H_2SO_4 - H_2O$, the only solid sulfate capable of isolation at 20° C., was the acid salt $C_5H_5N \cdot H_2SO_4$, and that a solution of pyridine, sulfuric acid and water in any proportions yields on evaporation $C_5H_5N \cdot H_2SO_4$ or a mixture of this salt and sulfuric acid. Assuming that the alkyl pyridines and quinoline would react similarly to pyridine, a series of sulfates was prepared by adding concentrated sulfuric acid to an excess of the base and cooling the reaction mixture. The sulfates of quinoline, 4-*n*-propyl-pyridine, 4-*n*-amyl-pyridine and 2,6-lutidine formed crystalline precipitates, which were recrystallized from alcohol and washed with acetone. The sulfates

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[2] Former Reilly Research Fellow and Professor of Agricultural and Biological Chemistry, respectively. This work was partially supported by a grant from the Reilly Tar and Chemical Corporation of Indianapolis, Indiana, and was carried out in the Department of Agricultural and Biological Chemistry in cooperation with the Department of Zoology and Entomology, and at the suggestion and with the cooperation of the Research Division of the Reilly Tar and Chemical Corporation.

[3] The results presented in this paper are taken from a thesis submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree, The Pennsylvania State College, December 1942.

of 2-ethyl-pyridine, 2-*n*-hexyl-pyridine and 2-mixed-undecyl-pyridine did not form precipitates but separated as viscous, water-soluble liquids.

In addition to its ability to form salts with mineral acids, pyridine also forms complex compounds with metallic salts. The formation of many such double salts has been discussed in detail by Lang (1888) and Pincussohn (1897). Following the general methods of these workers, and again assuming that the alkyl pyridines and quinoline would react similarly to pyridine itself, a series of copper chloride double salts was prepared by adding 50 grams of the free base to the calculated amount of copper chloride, dissolved in 200 ml. of alcohol. Crystalline precipitates formed in each case. These crystals were of the general type $(RC_5H_4N)_2 \cdot CuCl_2$, containing two molecules of the pyridine derivative to one of the metallic chloride,

Table 1.—Number of days required to kill 50 per cent of adult flour beetles.
(Ration Containing 10 Per Cent of Toxicant)

TOXICANT	DAYS
2 ethyl-pyridine sulfate	30
4-*n*-propyl-pyridine sulfate	55
4-*n*-amyl-pyridine sulfate	53
2-*n*-hexyl-pyridine sulfate	70
2 mixed undecyl-pyridine sulfate	100
quinoline sulfate	80
nicotine sulfate	66
check (no appreciable mortality after 100 days)	

since the calculated amount of $CuCl_2$ reacted completely with the pyridine derivatives.

In addition to the sulfate and the copper chloride double salt mentioned above, three crystalline salts—a picrate, a salicylate, and a zinc chloride double salt—were made of 4-*n*-propyl-pyridine. The zinc chloride salt was prepared by adding the free base to the calculated amount of zinc chloride, dissolved in water, and was apparently of the same type as the copper chloride salt. In the preparation of the picrate and the salicylate, however, it was assumed that the base, like the parent compound, would react with one molecule of the acid (Allen, 1912). The picrate was prepared by adding the base to an aqueous solution of the acid, and the salicylate by adding the base to an alcoholic solution of the acid.

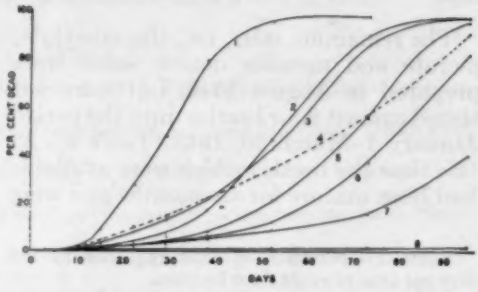

FIG. 1.—Effect of alkyl pyridine sulfates and related materials as stomach poisons against *Tribolium confusum*. Whole wheat flour 90%; test materials 10%.
1. 2-ethyl-pyridine sulfate
2. 4-*n*-amyl-pyridine sulfate
3. 4-*n*-propyl-pyridine sulfate
4. nicotine sulfate
5. 2-*n*-hexyl-pyridine sulfate
6. quinoline sulfate
7. 2-mixed-undecyl-pyridine sulfate
8. whole wheat flour controls

EXPERIMENTAL PROCEDURE AND RESULTS.—For each treatment, the required amount of crystalline toxicant was ground in a mortar and added to enough whole wheat flour to make 50 grams of ration. After mixing with a spatula, the ration was ground in a small Wiley mill to insure thorough mixing and crushing of all particles. The three test materials that were not crystalline were dissolved in alcohol and mixed with the flour while in solution. The solvent was evaporated off with frequent stirring of the mixture and the dried ration ground and mixed thoroughly. The mixture was divided into five 10-gram portions, each of which was placed in a 125 ml. beaker. One hundred adult flour beetles were added to each of these. The dead beetles were removed and counted, daily at first, but less frequently as the experiment progressed. The averages of the 5 replicates of each treatment, when plotted on coordinate paper, formed a smooth time-mortality curve, from which the time required to kill 50 per cent of the beetles was read without difficulty. This period of time was used as the standard of comparison for the various treatments considered.

The alkyl pyridine sulfates discussed above were prepared and tested during the period August 1 to October 30, 1942. At the start of the experiment the test beetles had been mature about two months, and were probably at the height of their resistance. The results of this test are given in table 1 and in figure 1.

The remaining salts, i.e., the salicylate, picrate and metallic double salts, were prepared in August 1942, but were not tested against flour beetles until the period January 1–March 30, 1943 (Table 2). At this time the beetles which were available had been mature for six months and were not as resistant as those used in the earlier test, the decrease in vigor being quite apparent in a comparison of the check groups of the two tests. For this reason, direct comparisons between the two tests are not justifiable. Each test must be considered complete in itself and it must be remembered that the values obtained are relative values only, and are dependent upon the vigor of the insect culture at the time of the experiment.

Table 2.—Number of days required to kill fifty per cent of adult flour beetles.

CONCENTRATION OF TOXICANT	10%	20%	40%
4-*n*-propyl-pyridine sulfate		25	18
4-*n*-propyl-pyridine salicylate	11	10	10
4-*n*-propyl-pyridine picrate	9	9	9
4-*n*-propyl-pyridine zinc chloride	15	13	10
4-*n*-propyl-pyridine copper chloride	8	8	8
2 ethyl-pyridine copper chloride	9	9	8
4-*n*-propyl-pyridine copper chloride	8	8	8
4-*n*-amyl-pyridine copper chloride	9	9	9
2,6-lutidine copper chloride	9	8	6
quinoline copper chloride	10	9	8
starvation check	*8*		
Whole wheat flour alone	*22*		

DISCUSSION.—Although none of the sulfates listed in table 1 proved to be highly toxic against the confused flour beetle, it is obvious that marked differences existed within the series of compounds. The sulfate of 2-ethyl-pyridine, for example, was much more toxic than the other salts of this series. The sulfates of 4-*n*-amyl-pyridine and 4-*n*-propyl-pyridine were next in order of toxicity, being very nearly equal to each other. The sulfate of 2-*n*-hexyl-pyridine was much less toxic than the above salts and 2-mixed-undecyl-pyridine sulfate, the last of the series, was the least toxic compound studied. While it is impossible in an experiment of this kind to distinguish clearly between toxicity due to contact action and that produced by the ingested toxicant, the former is in general much more rapid than the latter. The curves obtained in this experiment indicate that the toxicity of the compounds tested was largely, if not entirely, due to stomach poison rather than contact action. The most important observation to be drawn from this experiment is that the order of toxicity of the series of compounds was just the reverse of the order obtained by the authors (1943) in contact spray experiments against the greenhouse red spider, probably indicating that the shorter chain compounds were more effective stomach poisons while the longer chain compounds were more effective contact sprays.

The results given in table 2 appear to indicate that many of the compounds were repellent to the beetles. The entire series of copper chloride double salts, for example, gave time-mortality curves not significantly different from each other or from the starvation check. It is significant, also, that the increase in concentration of toxicant from 10 per cent to 40 per cent did not have any appreciable effect on the mortality—another indication that a condition of more or less complete repellency obtained.

The results given by the various salts of 4-*n*-propyl-pyridine demonstrate to what an extent the inherent toxicity of the base may be modified by the rest of the molecule. The copper chloride double salt was completely repellent and the picrate, salicylate, zinc chloride double salt and sulfate gave results that may be taken as an indication of medium toxicity, medium repellency or both. With the available data, it is impossible to be sure of the exact nature of the action of these materials for which the time-mortality curves fall between those of the starved controls and those of the fed controls.

SUMMARY.—Seventeen salts of various pyridine derivatives were prepared and tested against adults of the confused flour beetle, *Tribolium confusum* Duval. A series of alkyl pyridine sulfates gave results indicating that the salts with short alkyl side chains were more toxic than those with long chains. The toxicity of these compounds appeared to be due largely to the material ingested, rather than to contact action. A series of alkyl pyridine copper chloride double salts appeared to be completely repellent to the beetles. Results obtained with three additional salts of 4-*n*-propyl-pyridine indicated that the picrate, salicylate and the zinc chloride double salt were slightly toxic or partially repellent, or both.

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Relation of Chemical Constitution of Some N-Heterocyclic Compounds to Toxicity to *Aphis rumicis*[1]

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A number of workers have studied the contact action of pyridine and picoline on aphids. The work of Tattersfield & Gimingham (1927), Richardson & Shepard (1930) and Richardson & Smith (1923) indicated that these materials were of little value as aphicides. The authors have found no tests of the higher members of the alkyl pyridine series reported in the literature. By courtesy of the Reilly Tar and Chemical Corporation, samples of a number of higher alkyl pyridines and other pyridine derivatives have been made available for testing. The data reported in this paper are the results of aphicidal tests of 37 of these materials (See Table 1). From this group of compounds, it was possible to separate three distinct homologous series with alkyl chains ranging from one to eight carbon atoms in length.

PREPARATION OF MATERIALS.—With the exception of 2-amino-pyridine, which was crystalline in nature, all of the materials were liquids at room temperature. The lower members of the series were readily soluble in water but the higher members were progressively less water-soluble and required some emulsifying agent to make suspensions suitable for testing. For uniformity *Orvus* wetting agent (a sodium lauryl sulfate paste—see Cupples 1940) was used in all spray suspensions at the rate of .025 gm. of paste per 100 ml. sample. This acted as a wetting agent in all samples and as an emulsifier where necessary.

PROCEDURE OF REARING AND TESTING.—A stock culture of bean aphids, *Aphis rumicis*, L., was maintained in the greenhouse on potted nasturtium plants. A few (in most cases three) days before testing, aphids were transferred from the stock culture to small nasturtium plants in pots. Each test pot contained two plants, with

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[2] Former Reilly Research Fellow, Professor of Agricultural and Biological Chemistry and Assistant Professor of Economic Entomology, respectively. This work was partially supported by a grant from the Reilly Tar and Chemical Corporation, of Indianapolis, Indiana, and was carried out in the Department of Agricultural and Biological Chemistry and the Department of Zoology and Entomology, at the suggestion and with the cooperation of the Research Division of the Reilly Tar and Chemical Corporation.

an average over the entire experiment of 175 aphids per plant. The spraying was carried out in the spray chamber described by the senior authors (King & Frear 1943) in an earlier publication. For this experiment the chamber was moved into the laboratory, which was maintained at a more uniform temperature than the

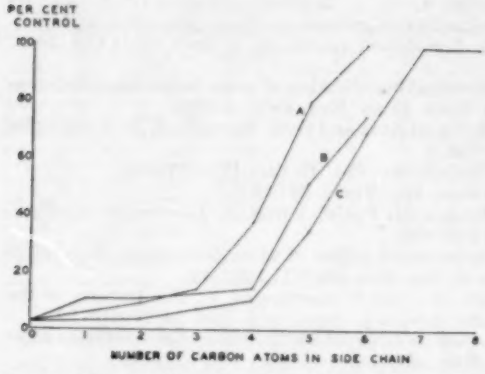

FIG. 1.—Effects of length and position of side chain on toxicity of alkyl pyridines to *Aphis rumicis*, L. Toxicant 1 per cent; *Orvus* wetting agent 0.025 per cent.

A. 4-*n*-alkyl-pyridines
B. 2-*n*-alkyl-6-methyl-pyridines
C. 2-*n*-alkyl-pyridines

greenhouse, and the test plants, after being sprayed to the point of run-off, were placed in a constant temperature room maintained at 28±1° C. This procedure eliminated much of the variability experienced in preliminary tests conducted in the greenhouse. Counts were made as described by Hartzell & Wilcoxon (1932).

EXPERIMENTAL DESIGN AND RESULTS.—Each material was tested at a concentration of 1.0 per cent, with 1.0 gm. of toxicant, 0.025 gm. of *Orvus* wetting agent paste and 99.0 ml. of distilled water per sample. Because of the large number of materials, it was impossible to complete a replicate on any given day. Approximately a third of the materials were tested each day, and the compounds were regrouped at the completion of each replicate to avoid an accumulation of errors caused by day-to-day variation in the aphid culture. With the exception of 2-amino-pyridine, of which only a very limited quantity was available, each material was tested on from 3 to 7 different days, depending on the degree of agreement obtained. As a control, a solution of *Orvus* in water was included in each day's run. With this as a reference, Abbott's per cent control (Abbott 1925) was calculated for each day's results. The averages obtained are tabulated.

DISCUSSION.—The most important results obtained in this experiment are summarized graphically in figure 1. In this figure, three homologous series of compounds are represented: a series of 2-*n*-alkyl-pyridines, a series of 4-*n*-alkyl-pyridines, and a series of 2-*n*-alkyl-6-methyl-pyridines. Comparison of the three curves shows at once that the 4-substituted alkyl pyridines were more toxic than the corresponding 2-substituted compounds.

Table 1.—Effects of sprays containing 1 per cent of various pyridine derivatives on *Aphis rumicis* L. and on nasturtiums.

MATERIAL	NO. OF DAYS TESTED	NO. OF APHIDS COUNTED	PER CENT CONTROL OF APHIDS	INJURY TO PLANTS
Pyridine	11	3438	2.4	none
2-methyl-pyridine	5	2193	2.9	none
2-ethyl-pyridine	5	2130	3.5	none
2-*n*-propyl-pyridine	3	1388	7.0	none
2-isopropyl-pyridine	5	1678	10.1	none
2-*n*-butyl-pyridine	3	1180	10.1	none
2-*n*-amyl-pyridine	4	1469	34.2	severe
2-*n*-hexyl-pyridine	4	1443	68.7	very severe
2-mixed-hexyl-pyridine	5	1728	53.3	severe
2-*n*-heptyl-pyridine	3	1055	98.2	very severe
2-*n*-octyl-pyridine	3	1032	97.4	very severe
2-(3-octyl)-pyridine	3	978	79.3	very severe
2-(2-me-octyl)-pyridine	3	799	97.5	very severe
2-(6-undecyl)-pyridine	4	1312	86.9	very severe
2-mixed-undecyl-pyridine	3	972	78.6	very severe
2-ethanol-pyridine	3	917	4.5	moderate
2-amino-pyridine	2	523	50.7	severe
3-methyl-pyridine	5	1891	8.4	none
4-ethyl-pyridine	3	1355	9.1	none
4-*n*-propyl-pyridine	4	1510	14.0	none
4-isopropyl-pyridine	4	1546	15.5	none
4-*n*-butyl-pyridine	4	1650	37.9	moderate
4-*n*-amyl-pyridine	5	2756	79.8	severe
4-(3-pentyl)-pyridine	3	1101	36.1	moderate
4-*n*-hexyl-pyridine	3	805	99.2	very severe
4-mixed-hexyl-pyridine	3	842	98.5	very severe
2-me-4-me-pyridine	4	1409	18.1	none
2-me-5-me-pyridine	5	1881	20.5	none
2-me-6-me-pyridine	7	2742	10.8	none
2-et-6-me-pyridine	7	2887	10.2	none
2-butyl-6-me-pyridine	5	1606	14.3	none
2-amyl-6-me-pyridine	7	3165	51.1	severe
2-hexyl-6-me-pyridine	4	1074	74.3	very severe
2-amino-6-me-pyridine	4	1255	58.5	severe
Quinoline	3	1057	76.4	very severe
2-*n*-hexyl-piperidine	3	1484	40.2	slight
4-*n*-amyl-piperidine	4	1101	81.7	severe

Likewise, they were more toxic than the 2, 6-, disubstituted derivatives. The general shape of the three curves indicates that regardless of the position of the substitution the toxicity of an alkyl pyridine increases markedly when the alkyl chain is increased to 5 or more carbon atoms. In the series tested this increase in toxicity was found to continue to the end of the series. The apparent peak shown in figure 1 for 2-*n*-heptyl-pyridine was proved to

be within the limits of experimental error and replicated tests of 2-*n*-heptyl-pyridine and 2-*n*-octyl-pyridine at a concentration of 0.25 per cent indicated that at this lower concentration the octyl derivative was definitely more toxic than the heptyl compound, the two materials giving 84.4 and 65.9 per cent control, respectively.

The shape of the curves also indicates that the addition of a $-CH_2-$ group to the side chain attached to the pyridine nucleus did not always produce the same increase in toxicity. At the lower end of the 2-*n*-alkyl-pyridine series, for example, the increase from one to two carbon atoms in the side chain (methyl to ethyl) increased the toxicity less than 1 per cent, while higher in the same series, the same increase (from amyl to hexyl) increased the toxicity 34 per cent. Similar relations may be observed in the other two series of compounds studied. The effect on toxicity of the methyl group in the 6 position in the 2-*n*-alkyl-6-methyl-derivatives was consistently low throughout the entire series.

It was observed in this series that toxicity was roughly parallel to insolubility in water, the more highly effective materials being those which formed emulsions with water.

A number of interesting points may be noted in connection with the miscellaneous materials which could not be grouped in any series. For example, the normal or straight-chain alkyl pyridines were generally more toxic than the corresponding branched-chain isomers. The propyl compounds were exceptions to this generalization, since the isopropyl derivatives were more toxic in each case than the normal isomers. Among the higher homologs, however, it will be seen that 2-*n*-hexyl-pyridine was more toxic than 2-mixed-hexyl-pyridine; 2-*n*-octyl-pyridine was more toxic than 2-(3-octyl)-pyridine; and 4-*n*-amyl-pyridine was more toxic than 4-(3-pentyl)-pyridine.

The amino derivatives, 2-amino-pyridine and 2-amino-6-methyl-pyridine are other interesting compounds which do not fit any series. The results given by these materials illustrate the marked increase in toxicity caused by the addition of an amino group to the pyridine nucleus, as compared to the slight effect of the addition of a methyl group. The high toxicity of the amino pyridines was noted by Wibaut (1934) and was reported by King & Frear (1943) in a study of the toxicity of these materials to the greenhouse red spider.

It was expected that 2-*n*-hexyl-piperidine and 4-*n*-amyl-piperidine would be more toxic than the corresponding pyridines, since Tattersfield & Gimingham (1927) as well as Richardson & Shepard (1930) found that hydrogenation of certain N-heterocyclic compounds increased toxicity markedly. In the present study, however, hydrogenation appeared to have no effect or to decrease toxicity, rather than to increase it.

A comparison of the three isomeric lutidines, or dimethyl pyridines, provides further proof that toxicity is enhanced more by alkyl substituents on the 3, 4 or 5 positions than on the 2 or 6 positions, since 2,4-dimethyl-pyridine and 2,5-dimethyl-pyridine gave twice as high control as 2,6-dimethyl-pyridine.

It is interesting to note that phytotoxic effect closely paralleled aphicidal action. The highest control obtained without injury to the plants was 20.5 per cent, given by 2,5-dimethyl-pyridine. 2-*n*-hexyl-piperidine gave 40.2 per cent control with only slight injury to the plants. All other compounds giving more than 20.5 per cent control of the insects caused burning of the foliage ranging from moderate to severe.

SUMMARY.—Pyridine and 36 related compounds were tested as contact insecticides against *Aphis rumicis*, L. In a series of 2-*n*-alkyl-pyridines it was found that compounds with 5 or more carbon atoms in the side chain were much more toxic than those with short chains, the toxicity increasing with the length of the substituent group. A series of 2-*n*-alkyl-6-methyl pyridines gave results closely parallel to those given by the 2-*n*-alkyl-pyridines. A series of 4-*n*-alkyl-pyridines was more toxic than the corresponding 2-*n*-alkyl-compounds. Normal or straight-chain alkyl compounds were generally more toxic than the branched chain isomers. The addition of an amino group to the pyridine nucleus increased the toxicity much more than the addition of a methyl group in the same position. Hydrogenation of two alkyl pyridines to the corresponding piperidines did not increase the toxicity. Phytotoxicity was generally parallel to aphicidal action.—4-6-44.

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Mercury Substitutes for Cabbage Maggot Control[1]

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In the spring of 1943 the scarcity and high price of mercury emphasized the desirability of testing a number of mercury substitutes for control of the cabbage maggot, *Hylemia brassicae* (Bouché). Tar paper pads (Goff 1891, Slingerland 1894, Schoene 1914, Hodgkiss & Horsfall 1922), liberal applications of tobacco dust (O'Kane, Cleveland & Hadley 1923, Glasgow 1925) and screening (Schoene 1908, Gibson & Treherne 1916, Parrott & Glasgow 1917) also have been recommended. Under present conditions in Pennsylvania none of these appeared practical.

METHODS AND MATERIALS.—Since facilities were limited and it was necessary to test a considerable number of materials in a small space with some degree of accuracy, cabbage plants could not be used. Radishes offered the most practical solution of the problem, since they required little space and are preferred hosts of this insect. The most apparent disadvantage is that radishes are injured by some materials which may safely be used on cabbage.

Two field tests were conducted. In the first experiment white icicle radishes were used and the dust treatments were made when the first pair of primary leaves were developing. This was on May 19 and 20. The dichloroethyl ether was applied on May 23. Scarlet globe radishes were used in the second experiment. These radishes had been planted earlier than those in the first experiment. On May 24, when these treatments were made, there were two pairs of primary leaves with the third pair beginning to show.

Only one application was made, and with the exception of corrosive sublimate and dichloroethyl ether, all treatments were used as dusts. Calomel was prepared as a mechanical mixture with hydrated lime and with bentonite. All the remaining dusts were made by dissolving the toxicant, mixing with bentonite, evaporating to dryness, and grinding. In the first experiment the treatments were replicated six times, while in the second there were seven replications. The plots were 7 feet in length and were randomized to eliminate any differences in location. In addition to the replicated treatments, preliminary trials were made in the first experiment on eight other materials, using a reduced number of plots.

The dust was applied from an attachment to a plunger type 2-quart duster. This was made by attaching a 3-foot length of half-inch heavy rubber tubing to a glass tube which extended several inches through a large rubber stopper. Through another hole in the stopper was inserted a 7 mm. bent glass tube which was the discharge outlet. The dust to be used was put into a pint jar which the large rubber stopper fitted. The rubber tube was attached to the exit opening from

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the duster where the discharge pipe had been removed. This arrangement eliminated the problem of cleaning the duster and 2 persons could apply the treatments quite rapidly.

RESULTS.—Since the per cent infestations of the treatments were not all between 20 and 80 per cent, the equivalent angle was used in the statistical treatment of the data.

Analysis of variance (Snedecor 1940) was made and is given in tables 1 and 2.

When applied early, a 10 per cent calomel dust was the best insecticide used which did not injure the radish plants. When it was applied late, as in the second experiment, it was found to be much less effective. With the same carrier there was not a significant difference between a 4 and a 10 per cent calomel dust but when a 4 per cent calomel dust with hydrated lime as the carrier was compared with a 10 per cent dust with bentonite the difference was significant. Calomel at 4 and at 10 per cent with bentonite as the carrier was more toxic than when used at the same strength with hydrated lime. When applied late, corrosive sublimate solution was found to be more toxic than 10 per cent calomel with hydrated lime as the carrier, but not significantly more toxic than 10 per cent calomel with bentonite as the carrier. The best insecticide tested late, which did not cause injury, was dichloroethyl ether.

The plots to which paradichlorobenzene, hexachlorethane, phenothiazine, 4-n-propyl-pyridine sulfate, 4-n-amyl-pyridine sulfate and quinoline sulfate were applied were not significantly better than those which had not been treated. Naphthalene at 40 per cent in the second experiment, 2-mixed-undecyl-pyridine sulfate, and calcium sulfide showed significant toxicity, but fell far short of giving satisfactory results.

Beta-naphthol and cresylic acid dusts, at the concentration used, killed the plants. Therefore no reliable information on toxicity to the insects was obtained. Limited trials of potassium sulfide, chlo-

Table 1.—Cabbage maggot tests on white icicle radishes.

(Experiment 1)

TREATMENT	INJURY[1]	% NOT INFESTED	SUM OF EQUIVALENT ANGLES
Calomel 10%	0	84.7	406.26
Paradichlorobenzene 10%	1	62.4	317.28
Paradichlorobenzene 20%	1	45.1	251.56
Paradichlorobenzene 40%	2	48.8	265.69
Dichloroethyl ether 1-400	0	74.8	362.14
Dichloroethyl ether 1-800	0	65.5	327.23
Naphthalene 10%	1	54.6	286.02
Naphthalene 20%	2	58.0	300.58
Naphthalene 40%	2	55.3	289.07
Check (no treatment)	0	50.1	270.69
2-ethyl-pyridine sulfate 10%	5	94.6	492.74
2-n-hexyl-pyridine sulfate 10%	3	74.3	374.39
2-mixed-undecyl-pyridine sulfate 10%	3	73.4	355.42
4-n-propyl-pyridine sulfate 10%	4	65.4	325.75
4-n-amyl-pyridine sulfate 10%	3	58.8	301.32
Quinoline sulfate 10%	2	67.4	332.71
Hexachlorethane 10%	1	58.2	298.62
Hexachlorethane 20%	2	47.0	259.59
Hexachlorethane 40%	2	49.8	269.19
Phenothiazine 10%	3	56.0	292.17
Phenothiazine 20%	4	58.8	301.70
Mathieson Alkali Weed Killer 10%	2	62.8	(2 plots)
Koppers Dormant Tar Oil 10%	5	100	(2 plots)
Beta naphthol 10%	D	—	(2 plots)
Pentacapryl laurylamine tripolyphosphate 10%	2	60.3	(2 plots)
Kerosene phosphine sulfide 10%	0	48.3	(2 plots)
Chlorophenyl thiophosphonic acid 10%	2	47.3	(2 plots)
Cresylic acid 10%	D	—	(4 plots)
Potassium sulfide 10%	1	68.1	(2 plots)

Analysis of variance on white icicle radishes

SOURCE OF VARIATION	DEGREE OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F
Totals	125	20,076.65	—	—
Treatments	20	10,691.18	534.56	6.08**
Replicates	5	598.79	119.76	1.36
Experimental error	100	8,786.68	87.87	—

** Significant at the 1 per cent level.

Difference between sums of the equivalent angles necessary for significance at the 5 per cent level = 64.5.

[1] Injury: 0, none; 1, very slight; 2, slight; 3, moderate; 4 severe; 5, very severe; D, dead.

Table 2.—Cabbage maggot tests on scarlet globe radishes.

(Experiment 2)

TREATMENT	INJURY[1]	% NOT INFESTED	SUM OF EQUIVALENT ANGLES
Calomel 4% (bentonite)	0	56.9	343.37
Calomel 10% (bentonite)	0	65.9	381.22
Calomel 4% (hydrated lime)	0	44.6	293.33
Calomel 10% (hydrated lime)	0	50.6	317.35
Dichloroethyl ether 1-200	0	89.1	501.65
Dichloroethyl ether 1-400	0	80.5	447.98
Corrosive sublimate 1-1000	2	74.1	419.62
Check (no treatment)	0	41.7	281.28
Phenothiazine 40%	3	46.0	298.80
Naphthalene 40%	1	67.0	386.27
Paradichlorobenzene 40%	1	48.5	307.44
Hexachlorethane 40%	1	48.5	308.65
Koppers Dormant Tar Oil 5%	3	70.8	402.28
Calcium sulfide 10%	0	56.6	342.19

Analysis of variance.

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F
Totals	97	12,047.81	—	—
Treatments	13	8,068.94	620.69	19.28**
Replicates	6	1,467.05	244.51	7.59**
Experimental error	78	2,511.82	32.20	—

** Significant at the 1 per cent level.

Difference between sums of the equivalent angles necessary for significance at the 5 per cent level = 42.3.

[1] Injury: 0, none; 1, very slight; 2, slight; 3, moderate.

rophenyl thioposphonic acid, kerosene phosphine sulfide, pentacapryl laurylamine tripolyphosphate and *Mathieson Alkali Weed Killer* gave no indication that further tests should be conducted with these materials.

The 2-ethyl-pyridine sulfate was found to be very effective in preventing insect injury, but caused serious damage to radish plants. A limited trial of a dust containing 10 per cent *Koppers Dormant Tar Oil* gave similar results. In experiment 2, where the *Koppers Dormant Oil* was used at 5 per cent in replicated plots, it was found to have insecticidal value, but caused moderate plant injury. The 2-n-hexyl-pyridine sulfate also was found to be of value in reducing maggot damage, but unsafe to use on radishes. These three new materials, which had been found to be most effective in preventing maggot damage to radishes, were given preliminary tests on cabbage. Ten per cent dusts were applied to the dampened stems of late cabbage before setting. Records kept for more than 2 months did not indicate any harmful effects.

SUMMARY.—Thirty-six different treatments, using 22 different materials, were applied to radishes under field conditions, and counts were made of cabbage maggot infestation. The new materials tested included several chlorinated hydrocarbons, N-heterocyclic compounds, and a number of miscellaneous substances. The best safe material tested early on radishes was a calomel-bentonite dust, while the best late treatment was with dichloroethyl ether. The smallest per cent infestation with maggot followed the use of a dormant tar oil and 2-ethyl-pyridine sulfate dust at 10 per cent, but injury to the radish plants was very severe. Several of the other pyridine derivatives were found to be quite effective, but caused considerable plant injury. Preliminary tests indicated that some of the treatments which caused serious injury to radishes, but were effective in preventing maggot damage can be used safely on cabbage plants. Further work is planned for the coming year. —6-23-44.

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J. C. GAINES

Mr. J. C. Gaines, of the Texas Agricultural Experiment Station is on leave of absence and is serving with the Sanitary Corps of the Army. Mr. Gaines is now Captain Gaines and is stationed in the India-China-Burma theatre of war according to latest reports.

Strength of Rotenone Dust Mixtures and Rate of Application in Pea Aphid Control

J. E. Dudley, Jr., and T. E. Bronson, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*[1,2]

Research for the purpose of developing effective control measures against the pea aphid, *Macrosiphum pisi* (Kalt.), infesting canning peas has been carried on at the Madison, Wis., field station for many years. After the efficacy of rotenone-containing roots against this insect was discovered in 1935 and 1936, experimental procedure began to be devoted to improvements in the formulas of rotenone-containing dust mixtures and sprays and in the technique of their application. The following experiments are concerned with dust mixtures only.

Among those recommending rotenone as a control for the pea aphid, questions have arisen as to the proper percentage of rotenone to be used in a dust mixtureand the most desirable quantity of dust to apply per acre, and also whether an increase in one would compensate in effectiveness for a comparable decrease in the other. Field experiments were therefore begun in 1942 in an effort to answer these questions.

Wilcox (1943), in experimenting with cryolite dust mixtures against the tomato fruitworm, found that undiluted cryolite (90 per cent) was approximately of the same effectiveness as dust mixtures containing 35, 50, and 70 per cent of cryolite, when the applications were made at the same dosage of sodium fluoaluminate per acre. When, however, he applied a mixture containing 70 per cent of cryolite at rates of 30, 60, 90, 120, and 150 pounds per acre, the effectiveness of the mixture was correlated with the rate. In both instances it would appear that the important factor was the quantity of sodium fluoaluminate applied per acre.

Methods.—Materials were applied by means of a power duster equipped with a 20-nozzle boom completely enclosed in canvas with a 45-foot rope-weighted trailer or apron attached. The boom covered a swath 24 feet wide. The truck hauling the duster traveled at the average rate of 2.5 miles per hour. Each treatment involved a single application of derris dust mixture. The treatments were assigned to the plots by the randomized-block method, there being 5 replicates in the 1942 experiment and 6 in 1943.

In determining the infestation a single sweep with an insect net was made in 5 widely separated parts of each plot on each sampling date and records were made of the numbers of living aphids caught.

Derris root powder containing by analysis 6.7 per cent of rotenone and 19 per cent of total extractives[3] was employed in the preparation of all the dust mixtures. In 1942, 2 per cent of soybean oil was used as the conditioning agent with pyrophyllite as the diluent. In 1943 the conditioning agent was 2 per cent of a straight-cut paraffin-base oil having a viscosity of 70 to 75 seconds Saybolt with talc as the diluent.

Experiment of 1942.—In 1942 it was not possible, because of sudden rainstorms, to treat all the replicates on the same day, therefore three were treated on June 3 and two on June 5. During application the wind velocity ranged from 5.5 to 10 miles per hour and the air temperature from 75° to 83° F., the temperature remaining moderately high throughout the period of observation from June 3 to 12. During this period rain, totalling 3.12 inches, fell on five days.

The Perfection variety of peas was used in the experiment. At the time of treatment the plants were not in blossom, ranged from 7 to 12 inches in height, and were infested with a rapidly increasing aphid population averaging 1,000 aphids per sweep of a net. Plots were slightly less than one-fourth acre in size. No untreated check plots were provided.

The limitations of the power duster employed made it impossible to apply the dosages accurately, but they ranged within 12 per cent of the dosages desired.

[1] In cooperation with the Wisconsin Agricultural Experiment Station.

[2] Credit is due the following people who assisted in the experiments: W. J. Reid, Jr., B. C. Fluke, Wayland Hull, and Miss Arlene Robinson.

[3] Analysis made by the Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine.

Results of this experiment are presented in table 1.

Results of the experiment of 1942 were not consistent. Analysis of the individual treatments (Table 1) reveals, however, that treatments 4, 5, and 6, upon both periods of observation, were superior only to treatment 1.

Arrangement of the treatments by combining into groups the first three and the last three, the dosage averaging 40 pounds per acre for each group, indicates that the application of 0.3 pound of rotenone per acre was superior to the application of 0.15 pound per acre.

Another arrangement of the data made by combining the treatments in which the quantity of dust mixture applied per acre was the same indicates that treatments 3 and 6 at a rate of 20 pounds per acre, but with the highest average rotenone content (1.125 per cent) were superior to treatments 1 and 4 at a rate of 60 pounds per acre, with only 0.375 per cent rotenone content.

Table 1.—Survival of the pea aphid following treatment with varying quantities of rotenone per acre. Experiment of 1942.

Treatment No.	Pounds of Rotenone per Acre		Rotenone in Dust Mixture	Pounds of Dust per Acre		Number of Aphids per Sweep After Indicated Number of Days	
	Desired	Applied		Desired	Applied	2 to 3	5 to 7
1	0.15	0.13	0.25%	60	53	24	20
2	.15	.13	.375	40	36	12	13
3	.15	.16	.75	20	22	12	9
4	.30	.28	.50	60	55	7	6
5	.30	.31	.75	40	42	16	11
6	.30	.33	1.50	20	22	8	6
Difference required for significance (at odds of 19 to 1)						7.6	6.3
1, 2, and 3	.15	.140	.458[1]	40	37	16	14
4, 5, and 6	.30	.306	.916[1]	40	40	10	8
1 and 4	.225	.225	.375[1]	60	54	16	13
2 and 5	.225	.220	.562[1]	40	39	14	12
3 and 6	.225	.245	1.125[1]	20	22	10	8

[1] These values are averages of those actually employed.

EXPERIMENT OF 1943.—In 1943 the previous experiment was enlarged and repeated. During application the air temperature ranged from 82° to 90° F. and remained moderately high while results were being recorded; the wind velocity, ranging between 9 and 17 miles per hour and averaging 12 miles, was rather high for effective dusting even with the use of a long trailer. The only rain during the experiment, amounting to 0.16 inch, fell the day after treatment.

Perfection peas from 10 to 12 inches in height and not yet in blossom were dusted on June 19. The aphid infestation was in a state of rapid increase, averaging 48 aphids per sweep on the day of the dust application, and in the checks 119 per sweep 2 days later and 650 per sweep 4 days later. As the available field of peas was larger than necessary for the 54 one-fifth-acre plots dusted, 6 check plots were left undusted so that the reduction in infestation of dusted plots compared with the checks might be determined.

A positive feeding device was developed by the junior author in the spring of 1943. This device, when mounted on the hopper of the power duster, made it possible to apply all the dust mixtures within 5 per cent of the desired rate. Results of the experiment are presented in table 2.

The results of 1943 (Table 2) were more consistent than were those of the previous year. Analysis of the individual treatments shows that treatments 3 to 9 were superior to 1 and 2 and that treatments 7, 8, and 9 were superior to 1, 2, 3, and 4.

By averaging the results of the treat-

ments in which the same number of pounds of rotenone were applied per acre, 3 groups were obtained in each of which an average of 40 pounds of mixture were applied per acre. This rearrangement indicates that the group with 0.30 pound of rotenone was superior to the group with 0.15 pound, which, in turn, was superior to the group with only 0.075 pound.

Rearrangement of the data by averaging the results of treatments in which the poundage of dust mixture applied per acre was the same indicates that the 20-pound rate was more effective than the 60-pound rate. This is of particular interest, as the same average dosage of rotenone was applied, the average rotenone contents being 0.875 and 0.292 per cent for the 20- and 60-pound treatments respectively.

A definite reduction in the efficacy of rotenone against the pea aphid takes place in oil-conditioned dust mixtures whose rotenone contents are somewhere between 0.375 and 0.25 per cent. This phenomenon has been observed by the authors many times in field tests. In the 1942 experiment (Table 1) this break is exemplified between rotenone contents of 0.375 and 0.25 per cent (treatments 2 and 1). In the experiment of 1943 (Table 2) the break occurs twice—once between rotenone contents of 0.375 per cent and 0.25 per cent (treatments 5 and 4) and again between rotenone contents of 0.375 per cent and 0.187 per cent (treatments 3 and 2). This marked decrease in the toxicity of dust mixtures with rotenone contents below 0.375 per cent is not compensated for by any *moderate* increase in the rate of application of dust mixtures. (Note treatments 3 and 4 of the 1943 experiment (Table 2). Treatment 4 was only a little more effective than treatment 3, yet in treatment 4 three times as much dust mixture and twice as much rotenone was applied per acre.)

Table 2.—Average mortality of the pea aphid following treatment with varying quantities of rotenone per acre. Experiment of 1943.

Treatment No.	Pounds per Acre		Percentage of Rotenone	Aphids per Sweep: 3 Observations[1]	Mortality in Dusted Plots
	Dust	Rotenone			
1	60	0.075	0.125	40	89.0%
2	40	.075	.187	40	89.0
3	20	.075	.375	24	92.0
4	60	.15	.25	21	94.0%
5	40	.15	.375	15	95.7
6	20	.15	.75	18	93.0
7	60	.30	.50	11	96.7%
8	40	.30	.75	11	96.7
9	20	.30	1.50	12	96.0
Difference required for significance (at odds of 19 to 1)				8	—
1, 2, and 3	40	.075	.229[2]	35	90
4, 5, and 6	40	.15	.458[2]	18	94
7, 8, and 9	40	.30	.916[2]	11	96.5
1, 4, and 7	60	.175	.292[2]	24	93
2, 5, and 8	40	.175	.437[2]	22	94
3, 6, and 9	20	.175	.875[2]	18	94

[1] Observations taken 2, 4, and 6 days after application.
[2] These values are averages of those actually employed.

CONCLUSIONS.—The following conclusions may be drawn from the two years' results: (1) When an oil-conditioned rotenone-bearing dust mixture is employed, the quantity of rotenone applied per acre governs, within limits, the resulting mortality of the pea aphid. (2) When as much as 0.30 pound of rotenone per acre is applied, it makes little difference whether a dust mixture of high rotenone content at a low rate is used or a dust mixture of lower rotenone content at a higher rate. When, however, only 0.15 pound or less of rotenone per acre is applied, a dust mixture of high rotenone content at a low

rate is preferable to a dust mixture of low rotenone content at a high rate. (3) A marked decrease in pea aphid mortality occurs in an oil-conditioned dust mixture when the rotenone content is decreased from 0.375 per cent to 0.25 per cent or lower.

From the results of 1943 it may be concluded that when either 0.15 pound or 0.30 pound of rotenone is applied per acre, a decrease in the rotenone content of the dust mixture may be partially compensated for by an increase in the rate of application, but that this relationship fails to hold true when only 0.075 pound of rotenone per acre is applied with dust mixtures of the rotenone contents used. —4-6-44.

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Laboratory Technique for Testing Insecticidal Dusts for Pea Aphid Control

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Regardless of the technique used for the relative evaluation of insecticides, considerable error may occur even when the greatest care is used in procedure. A procedure is given which, with the needed equipment for testing insecticidal dusts against the pea aphid, has been found to give highly satisfactory results over a period of 5 years.

With the number of variables that occur in insects, plants, materials and equipment, and the effect of temperature and humidity, it is often difficult to obtain comparable data in replicated tests even when the number of variables is reduced to a minimum.

To conduct a satisfactory testing program with insecticides, three primary conditions must exist.

1. The operator must have adequate equipment for preparing and applying uniform amounts of material by a standardized procedure.

2. A readily available supply of insects for testing is necessary to make an adequate number of tests so that comparable data can be obtained to make definite conclusions possible.

3. For laboratory experiments, greenhouse facilities must be available for maintaining temperature and humidity control.

A satisfactory method for rearing pea aphids in the greenhouse for laboratory testing has been worked out by Stone (1940). With slight modifications that method has been used successfully by a number of workers in the Department of Economic Entomology, University of Wisconsin.

Using this method a successive series of healthy and vigorous uniform plants can be obtained at almost any time. Reduction of natural light in December, Januaryand February makes this period less favorable for plant and aphid production. This difficulty can be corrected by the use of suitable artificial light. Holding greenhouse temperatures below 70° F. will aid in the production of vigorous plants and aphids.

It is possible to synchronize the supply of plants and aphids so that, with uniform greenhouse conditions, test schedules can be arranged ahead for a period of several months. A satisfactory testing procedure has been developed for securing consistent and comparable results with dusts under test for control of the pea aphid.

A dust applicator has been developed for the application of a uniform amount of dust to one or more plants at one time. When such dusts are blown through the applicator, measurable electrostatic charges of varying intensity are produced on the applicator and in the dust clouds. The degree of charge can be recorded by contacting the apparatus with an electrostatic voltmeter.

PRODUCTION OF PLANTS FOR APHID REARING.—Two types of plants were used: —stock plants on which the test aphids

were reared, and test plants on which a definite number of aphids were placed for testing the insecticidal dust. All plants were wilt-resistant Perfection peas.[1]

The stock plants were grown in 6- to 10-inch flower pots in a mixture of two-thirds compost and one-third sand. They were planted every 5 days so there would always be a sufficient supply of young succulent plants present for aphid rearing. When planting, the pots were filled about three-fourths full of the soil mixture and then covered with about a half-inch of clean sand. Pea seeds were treated with *Semesan Jr.* and were planted in approximately one-half inch of sand.

As soon as the plants emerged above the sand they were placed under artificial lights on a bench. Artificial lighting was necessary during the winter months for vigorous plant growth. In the greenhouse it took about 5 days for the plants to emerge. The planting schedule was maintained so that five different age groups were present from those just planted to plants old enough to be infested, Thus there were succulent plants available for aphid rearing about every 5 days.

In figure 1 the 20-day old plant at the right is the proper size for aphid infestation to rear test material. Ten to twelve plants at this stage can support from 100 to 150 aphids from the first nymphal stage to maturity. Since the young aphids are produced over a period of 24 hours and do not all mature at the same time, a 2-day margin exists in which to use the aphids for testing.

PRODUCTION OF PLANTS FOR APHID TESTING.—A single plant infested with 20 aphids was used for testing an insecticide. All plants were grown in No. 2 enamel cans, (Figure 1). Cans were usde for growing the test plants because they were easily kept clean, required less space than pots, were lighter to handle in groups, and were more suitable for use in temperature and humidity control chambers. The plant at the right, 20 days old, was the type used in the testing procedure.

In preparing a plant for use in testing it was watered a few hours before infesting. Then a disc of wax paper the same size as the inside of the can was placed around the plant on top of the damp earth and covered with dry sifted sand. The paper, when properly fitted, breaks the capillary column of water and prevents excessive evaporation from the surface, making it possible for the plants to go longer without watering. This also kept the top sand dry and aided materially when counting dead or unhealthy aphids that had fallen from the plant. Thus more consistent results were obtained for moist sand seems to influence the mortality of aphids when they are in contact with it.

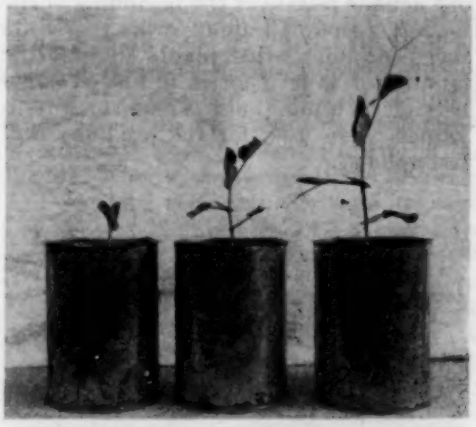

FIG. 1.—A series of three test plants in different stages of development. The plant at the right is 20 days old, the age used for testing purposes.

The leaves at the lower nodes near the sand were removed so that the aphids would settle higher on the plants and make them more accessible to the insecticide. Twelve to 24 hours before dusting, the test plants were infested with mature apterous agamic aphids from the stock plants. The aphids were removed by holding the stock plants over a shallow pan and blowing on them. The aphids then fell from the plants to the pan. By removing the aphids in this manner there is little danger of injuring their mouthparts, as might be done by picking or brushing them off. A desired number of mature aphids were removed to a dry 125 cc. erlenmeyer flask or a suitable container for checking the number. The aphids were then placed in an inverted screen cage and allowed to crawl onto the plant, with the plant in contact with the cage. The cages used were made of 20-mesh screen, 8 inches high and of such diameter that they fitted snugly inside a No. 2 can. This eliminated the danger of their being easily tipped off the plants

[1] English broad beans can be used for aphid rearing.

when handling. The infested test plants were allowed to set 12 to 24 hours before dusting to permit the aphids to become well established.

APHID REARING.—The rearing method was so developed that the aphids used would be of approximately the same age and vigor, for it has been found that aphids of different stages and ages show varying susceptibility to an insecticide.

The stock was started each fall from one agamic female at the beginning of the testing season. All aphids were reared on succulent uncrowded plants so that the number of winged forms produced could be kept at a minimum. These stock individuals were used for the production of test aphids.

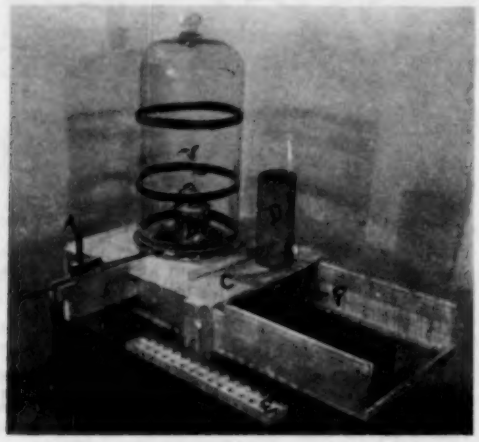

FIG. 2.—Dusting unit ready for attachment of pressure hose and dust application. *A*, Dust injector. *B*, Test plant. *C*, Base for holding injector, test plant and bell jar. *D*, Cage removed for dusting. *E*, Rack containing measured amounts of test materials. *F*, Movable metal slide for trapping excess dust.

In order to produce a large number of uniform test aphids, a uniform number of young agamic females just starting to reproduce was transferred to a series of the stock plants for a given length of time. The size and number of plants per pot determines the number of reproducing aphids to be used. Four to five aphids per plant were allowed to reproduce for about 12 hours and then removed without disturbing the young. The infestation was made by putting the reproducing aphids into a screen cage and placing the cage over the potted plants. In a short time the aphids moved from the cage to the plants. It also has been found important to have all the plants about the same size because uneven plant growth usually resulted in uneven aphid development. The conditions in the greenhouse determined the length of time necessary for the young aphids to reach the testing age. Under optimum conditions, 65 to 70° F. and 30 to 50 per cent relative humidity, pea aphid development takes about 7 to 8 days. The number of aphids per plant will influence the length of development and the size and vigor of the insects. Thus, it is very important to have uniform, healthy, uncrowded plants for the production of uniform and vigorous aphids.

DUSTING APPARATUS AND TECHNIQUE.—The dusting technique was developed for the application of an insecticidal dust to infested plants under laboratory conditions. The infested plants were dusted by placing them under a bell jar and injecting a weighed amount of dust into the bell jar by a specially designed dust injector.

The dust injector developed and used was based on the principle that a fast moving stream of air passing by an opening in a tube will suck air in through the opening. Taking advantage of this fact, a tube was fitted with a hopper in which the dust was placed, and when air was forced through the tube, the dust was sucked out of the hopper and blown into the bell jar.

The dust injector was made of a quarter-inch copper tube about 12 inches long and fitted with a dust hopper about 6 inches from the end. The hopper was made of half-inch copper tubing about 4 inches long and drawn to a fine point with a one-sixteenth inch hole in the tip. The tip was bent to nearly a right angle and inserted in a hole in the quarter-inch tube in such a way that it partially closed the tube. Thus the small tube is constricted and the air speed increases at the opening at the tip of the dust hopper. The outlet end of the dust injector was fitted with an elbow to direct the stream of dust upward. The dusting unit is shown in figure 2, C being the base for the bell jar and dust injector holder. It is necessary to have the dust injector held firmly in the same position at all times so the dust leaving the nozzle will hit the curved surface near the top of the bell jar and be distributed in all directions. The base for the jar has holes in the sides so there will

be no back pressure in the jar when the dust is blown in. This also favored better distribution of the dust on the plants.

Figure 3 shows equipment in position for dusting an infested plant. The plant is so placed that the dust stream from the duster does not strike it directly. The bell jar is 9 inches in diameter and 18 inches high. The dust may be applied with variable pressure and as soon as the dust is out of the hopper the stream of air is shut off. The plant may be allowed to remain under the bell jar for as long as desirable after the treatment is applied. With test plants and dusts ready, it is possible to dust one plant per minute. After each dusting operation the equipment should be cleaned by wiping with a dry cloth.

PREPARATION OF DUST MIXTURES.—All dusts to be tested are mixed by hand with mortar and pestle. The ground rotenone-bearing root is sifted through a 100-mesh soil sieve to remove all coarse fiber. The ground cube and the disperser are first blended and the liquid supplements added last. After the supplements are added the dusts are mixed and sifted through a 30-mesh screen until no aggregates remain. All dusts are resifted just before introduction into the dust ejector to break up any lumps which might plug the small opening in the bottom of the hopper. The dusts must be in good physical condition to pass through the duster.

TESTING PROCEDURE.—For each test, two to three plants are used, each plant being infested with 15 to 20 mature aphids. Mature aphids are used for testing because, as a group, they are more uniform than other stages. Results are observed at 24, 48, and 72-hour periods except with quick acting dusts when the first records may be taken in 12 hours. Some dust mixtures are rather slow acting and it is sometimes difficult to decide on mortality. After due consideration it was decided to base the control estimate on the number of aphids left on a plant at the end of the test period. Those which are alive and capable of crawling onto the plant, whether on or off the plants are considered to be on the plant. A group of tests may be run for 24 to 72 hours, depending upon the rapidity and completeness of the kill. If the mortality rate develops slowly, the tests should be run 72 hours. Tests can seldom be run longer because the check plants cannot stand the heavy infestation of young aphids produced. Suitable checks should always be maintained with all tests and be handled in the same way as the treated plants except that no insecticide is applied.

One-tenth to one-half gram of the material being tested can be used for each application. Since the dispersing agents vary in physical condition and in volume, dust mixtures do not flow through the injector equally well, and in some cases the physical characteristics of a dust causes the hopper to clog so that not all of the material passes through. Fortunately, this does not happen often, and if the dust is removed from the injector and rescreened, it will pass through the injector. When the injector is set properly and held in the same position for each application, similar degrees of coverage are secured. Because of even slight differences in plant condition, the kill on one plant in a series sometimes varies, but with a series of replicated tests, a good average value can be established for any dust mixture.

ACCURACY OF TESTING METHOD.—During a period of four years several thousand single plants infested with from 10 to 20 aphids have been used for testing insecticidal dusts against the pea aphid.

In the start of the investigation three plants each infested with 20 aphids were used for a single test. But as the investigation developed and increasing numbers of dust mixtures had to be tested at one time, it was not always possible with the facilities available to have sufficient numbers of suitable plants and aphids. Therefore the number of plants was dropped to two and the aphids to 15 per plant for preliminary tests. In order to determine the accuracy of the testing method, and also to learn the number of replicated tests necessary for obtaining reliable comparisons, selected test data were submitted to our Agricultural Statistical Service for a statistical analysis. The analyses of the following group were made by Professor J. H. Torrie and Miss Virginia Beal.

Analyses of variance were made on the data from four groups. All tests in Group 1 and the two talc series in Group 2 were made by R. L. Janes. The tests with *Perry Clay*, *Bancroft Clay* and *Baggo* were made by E. J. Campau. The tests in Groups 3 and 4 were made by H. F. Wilson.

Group 1. This series consisted of the data from five dispersing agents showing relatively high control with concentrations of 0.1, 0.25, 0.5, 0.75 and 1 per cent rotenone activated with 2 per cent SAE10 oil. The dispersants included *Pyrax ABB, Electro Calcium Carbonate, AA Muscovite* talc, a soapstone from a California deposit, and a Maryland talc. Each agent was tested five times with three plants each infested with 20 aphids.

Group 2. This series consisted of the data from five dispersants showing a wide variation in control with low concentrations of rotenone and a tendency to level off at 0.5 to 1 per cent rotenone. The dust mixtures contained the same concentrations of rotenone and oil used in the first group. The dispersants included *Eastern Magnesia Talc, Rlue Bidge Talc, Perry Clay, Bancroft Clay* and a finely ground plant material (*Baggo*). Each dispersing agent was tested five times with three plants, each infested with 20 aphids.

Group 3. This series consisted of the data from tests with five different grades of pyrophyllite mixed with 0.1 per cent rotenone and 2 per cent SAE10 oil. Each grade was tested five times with two single plants, each infested with 15 aphids.

Group 4. This series consisted of the data from a series of dust mixtures mixed in May, 1942 and containing 0.5 per cent rotenone and from zero to four per cent SAE10 lubricating oil sold by the Pure Oil Company in Fox Lake, Wisconsin. Each concentration of oil was tested five times with two single plants, each infested with 15 aphids.

The mean number of aphids for each test series is given in Table 1.

Analyses of variance were carried out on the data from each group separately.

Table 1.—Mean number of aphids surviving for each test and concentration of material.

	Per Cent Rotenone					Over all Means For Each Dust Concentration
	0.1	0.25	0.5	0.75	1.0	
Group I						
Pyrax	0.93	0.87	1.33	0.67	1.60	1.08
Calcium carbonate	1.13	1.0	2.0	1.73	1.73	1.52
AA Talc-Muscovite	2.40	2.47	1.80	1.93	1.80	2.08
Soapstone	3.0	1.47	1.20	1.40	1.0	1.61
Harford Talc	4.73	2.93	2.13	1.93	1.87	2.72
Group II						
Emtco Talc	6.40	4.53	2.13	2.27	2.53	3.57
Blue Ridge Talc	8.93	4.47	1.87	2.40	1.93	3.92
Baggo	9.27	2.73	1.07	1.87	2.27	3.44
Perry Clay	10.33	7.07	7.60	1.73	0.40	4.43
Bancroft Clay	14.07	14.07	10.67	4.20	2.80	9.16
Group III Grades of *Pyrax*						
	1	2	3	4	5	
Mean number of aphids	2.70	2.90	2.60	1.00	1.30	2.10
Group IV 0.5 per cent rotenone plus variable concentrations of oil.						
Per cent of oil	0	1%	2%	3%	4%	
Mean number of aphids	5.50	3.50	3.0	1 0	0.50	2.70

In interpreting the results two sources of variation were used, the sampling and experimental errors. The sampling error is the random variation between plants within a dust, concentration, and trial, whereas the experimental error measures the failure of the trials to be the same when the treatment effect has been removed. The experimental error has in it two independent sources of variation. The first is due to random variation of trials within a dust and concentration, and will be designated as A. The second is the variance of the mean of k plants about the mean of the trial and will be designated as B/k, where k is the number of plants per trial. The experimental error mean square in terms of trial means is therefore $A+B/k$, which, on the basis of an individual plant, would be $k(A+B/k) = kA+B$.

A study of the two parts of the experi-

mental error, according to the method given by Snedecor, made it possible to estimate the relative efficiency of various combinations of numbers of trials and plants per trial.

The estimated variance of a treatment mean on a single plant basis was obtained, $V_x=(kA+B)/kt$, for different numbers of plants (k) per trial and trials (t). The relative precision factor was calculated for various combinations of k and t, with $k=3$, $t=5$ as a base. The V_x of any particular combination was divided by V_x for $k=3$, $t=5$, and the result times 100.

A summary of the analyses showed that in Groups I and II the precision of the experiments was increased by increasing k or t, but that the precision of an experiment was bettered more rapidly by increasing t than k. In Groups III and IV it was found that the use of 15 aphids per plant was justified and very nearly as efficient as the use of 20. A good experimental procedure with thrifty plants and aphids, would consist of weeding out the obviously poor dusts by a less efficient experiment using 15 aphids per plant with $t=5$, $k=2$, and testing the remaining dusts more precisely with an experiment like $t=7$ or more and $k=2$.—4-10-44.

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Electrostatic Charge Effects Produced by Insecticidal Dusts

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In a greenhouse study of insecticidal dusts for the control of the pea aphid, a wide variation was found in the dispersion characteristics of finely ground nonmetallic minerals used as dispersing agents. It appeared that this dispersing factor had some relationship to the per cent control of the aphid in experimental tests.

It had been shown that all dispersing materials produced variable electrostatic charge effects when blown through a dust applicator. Some materials produced little or no charge while others produced from a medium to high charge. The degree of charge appeared to have some relation to the dispersion of the dusts to all parts of the plant and this in turn seemed to affect the degree of aphid control.

The principal cause for some differences in aphid control seemed to result from poor dispersion of dust particles to aphids feeding on the underleaf surface. If little or no charge was produced the dust particles tended to aggregate and did not give a fine dispersion of the underleaf surface. If a relatively high charge was produced the dispersion of finely divided dust particles on stems and the underleaf surface was greatly increased. Theoretically these conditions may prevail due to differences of electric potential in the dust cloud which in turn causes the charged particles to repel one another and to be drawn to the plant by the opposite induced charge. To obtain definite data on this phenomena a laboratory method for studying the electrostatic charge effects of variable dust mixtures has been developed. With this method the relative charge producing capacity of each material was determined.

EQUIPMENT.—The complete testing unit includes a dust blower, an electrostatic voltmeter, a dust collecting box and a compressor unit producing up to 75 pounds pressure.

The Dust Blower. The dust blower (Fig. 1) was made with two pieces of copper tubing about 0.6-inch in diameter. The dust delivery tube was 20 inches long with a rounded brass collar at each end to prevent corona effects so far as possible. A dust hopper 6 inches long and tapered to an opening about 0.2-inch in diameter at the bottom was soldered into the upper side of the delivery tube, 12 inches from the outlet. The lower end of the hopper was bent at an angle so that the opening pointed toward the outlet of the delivery tube. This helped to hold the dust from dropping onto the floor of the delivery

tube and permitted drawing the dust out by suction rather than by direct discharge. A series of indentations in the delivery tube beyond the hopper caused increased turbulence in the dust stream and more consistent charge records.

The blower was held in place by wooden clamps with rubber tubing at both points of contact for insulation. The charges were carried from the blower to the voltmeter by two sections of insulated wire. The first section of flexible wire was soldered on the delivery tube near the end. This wire connected with a heavier semi-rigid wire run part way through a hard rubber tube and bent at right angles to rest on the contact point of the voltmeter.

FIG. 1.—Delivery tube of apparatus for recording electrostatic charge effects of insecticidal dusts. A. Location of indentures in dust tube for creating dust turbulence. B. Contact point of wire leading to connection with voltmeter.

The hard rubber tube was fastened to a hinge at the top of a wooden stand. By using the outer end of the hard rubber tube as a handle the contact wire was brought against a rounded knob on the voltmeter when the charge was developed and removed as soon as the full force of the charge occurred.

Electrostatic Voltmeters. Two recording voltmeters were used in recording charges. Voltmeter *A* was calibrated to 6000 volts and was found to give satisfactory readings with charges as low as 100 volts. Voltmeter B was used whenever the charge for a dust was 1000 or more volts. It was calibrated to record up to approximately 14,000 volts. When charges greater than 14,000 volts occurred they were estimated by means of a calibrated spark gap.

Dust Collecting Box. The dust collecting box consisted of a wooden box 4 feet high and 12 inches square as a base and a cardboard box 14 inches square and 2 feet high as the top section. The cardboard box was cut out at the bottom so that it fitted over the wooden base and rested on wooden cleats. The original purpose of the cardboard box was to allow for expansion when the dust was blown into the box. The disposal of the blown dust was further taken care of by two rubber outlet tubes leading out of the wooden portion of the dust collector. This prevented back pressure and blowing of the dust out around the end of the dust delivery tube.

Compressor Unit. A simple compressor unit was used to provide air pressure and when the air pressure gauge showed a desired amount the air was released by opening a stop cock. A moisture-drying unit was set between the compressor and the pressure tank and a glass tube 6 inches long and paraffined on the inside to prevent current leakage was inserted between the hose connections leading from the compressor to the dust ejector.

METHOD OF PROCEDURE.—In making the tests parallel samples of 0.5 cc. quantities of material was first screened and then poured into the dust hopper. The wire conducting the charge from the blower to the meter was placed in contact with the meter head. The stop cock on the compressor was then opened and the contact wire removed when the needle of the meter indicated maximum force of charge.

The maximum electrostatic charge varied considerably between different materials, both as to the rapidity and the intensity at which it occurred. In talcs which were relatively slow to electrify, the maximum charge was slow in recording while with pyrophyllite the maximum charge was instantaneous. The charge on the meter would hold for considerable time so it was possible to let the needle come to complete rest before recording the reading. The meter and blower were discharged after each test by touching a ground wire to the different pieces of equipment. At the start of the study the blower was cleaned after each dust discharge, but it was found that handling and cleaning the blower caused widely separated readings. Readings were more consistent if the delivery tube was cleaned only between changes in material.

Each dispersing agent had to be tested on the basis of its peculiar physical qualities. Some materials flowed freely and gave consistent records with as few as five trials, others were "gummy" in nature and sometimes stuck in the hopper so it was necessary to make 10 to 20 readings to obtain satisfactory records.

A hard rubber rod and glass rod rubbed on silk or fur were used for the preliminary determination of the sign of the charge on the blower which was opposite to the charge in the dust cloud. However, to obtain definite indications of positive or negative sign, the contact wire was allowed to rest on the voltmeter knob and additions of the same or different dust samples were poured into the hopper without touching it. If the blower was not touched in loading, additional dust could be dropped into the hopper without changing the charge on the voltmeter. Then by blowing the same or different dusts through the blower the voltage was increased or decresased depending upon the sign of charge of the added dust. Materials of the same sign increased the voltage. Materials of opposite sign decreased the voltage reading.

RESULTS.—To what extent electrostatic charges may effect dust dispersion and insect control under field conditions is yet to be determined. The sign and charge was found to depend upon several factors. Particle size was an important factor, large particles, 40μ generally give high charges. Very fine particles, 2μ or less in size as are found in sedimentary clays, generally produced very low charges. Rapidity of dispersion, atmospheric conditions and quantity of materials are other important factors. In the laboratory the records were taken with temperatures between 80 and 85° F. and a relative humidity between 30 and 40 per cent. The voltage produced by any material was found to decrease with lower temperatures and increased with higher temperatures. Not much change in the voltage was observed with a relative humidity of 40 per cent or less. When the relative humidity was increased to 50 per cent the voltmeter either did not record a voltage or lost it too quickly for making satisfactory records.

Regardless of the sign of the charge, materials of the same nature reproduced charges of the same sign. Materials high in silica always gave a (+) charge on the blower and a (−) charge in the dust stream. Dusts of plant or animal origin always produced (−) charges on the blower and (+) charges in the dust stream.

AA Talc, calcium carbonate and gypsum gave charges opposite to those of talcs and pyrophyllite. Flower sulphur and various commercial arsenical, copper and iron compounds so far tested indicated (+) charges in the dust stream. When two materials with opposite signs of charge were combined the charge for the mixture was less than the charge for either original material, and when suitable combinations were made the charge was reduced to zero. In cases where two materials, one producing a high charge and one producing a medium charge, were combined, the sign of the material having the highest charge remained, but if continued amounts of the material having the opposite sign were added, the charge dropped to zero and then increased again but the sign of the charge was that of the increased material.

A number of samples of powdered commercial insecticides and fungicides were studied and it was found that a wide variation of capacity to produce electrostatic charges existed between materials presumed to have the same chemical composition. For example (Table 1) five different brands of lead arsenate gave charges from 0 to 5200 volts. Compounds containing fluorine showed a similar variation although different combinations of minerals could explain this difference.

The difference between five samples of flower sulphur and five samples of conditioned sulphur are also shown in table 1. Flower sulphur generally produced no charge at all, and when slight charges did occur, they were considered due to impurities in the sample. The sign of sulphur was indicated as weakly (+) since it depressed *Pyrax* when sulphur was used after *Pyrax*. However, *AA Talc* which produces a charge opposite to that of *Pyrax*, was also depressed by sulphur. Therefore the reduction in charge may have been due to the very small particle size of the sulphur. But when mixed lots of conditioned sulphur and *Pyrax* or *AA Talc* were tested, the behavior of the voltmeter indicated that the charge for sulphur was (+).

All samples of conditioned sulphur gave high charges and the dispersion was somewhat better than with flower sulphur but not significantly so. Since the charges for all samples of conditioned sulphur were of the positive sign and comparatively high, it can be assumed that the conditioning agents also produce (+) charges.

Five samples of mineral dusts containing fluorine were examined and found to produce charges from 0 to 10,000 volts. The sign of charge for each material was (+).

Table 1.—Electrostatic charges developed by insecticidal dusts composed entirely of mineral elements.

MATERIAL[1]	CHARGE IN VOLTS	RELATIVE DISPERSION[2]
Arsenicals		
Lead arsenate 1	5200	Good
Lead arsenate 2	1200	Fair
Lead arsenate 3	3000	Fair
Lead arsenate 4	700	Poor
Lead arsenate 5	0	Poor
Zinc arsenate	0	Poor
Calcium arsenate	8300	Excellent
Paris green	0	Fair
White arsenic	0	Poor
Flourine compounds		
Barium fluosilicate	2000	Poor
Calcium fluosilicate	0	Fair
Sodium fluos.licate	3000	Good
Sodium fluoaluminate	1000	Good
Sodium fluoride	5000	Good
Copper dusts		
Corona Bordeaux	4000	Good
Cuprocide	200	Fair
tri-basic copper dust	100	Fair
Sulphur dusts		
Flowers of sulphur 1	0	Poor
Flowers of sulphur 2	0	Poor
Flowers of sulphur 3	0	Poor
Flowers of sulphur 4	0	Poor
Flowers of sulphur 5 (Micronized)	100	Fair
Conditioned sulphur 1	4500	Good
Conditioned sulphur 2	4700	Excellent
Conditioned sulphur 3	4000	Good
Conditioned sulphur 4	4000	Good
Conditioned sulphur 5	5000	Good

[1] The samples used in making these records were standard brands furnished by manufacturers. All samples were run through a 325-mesh screen to obtain somewhat comparable samples. However, the average particle size for all samples was not the same. Some materials contained more small sized particles than others.

[2] Observations made by estimating number of particles on the underleaf surface of pea vines by counting particles in ¼-inch circles.

The sign of charge for each material was first determined by the use of glass and hard rubber rods although a glass rod is sufficient. Determinations considered more accurate were made by mixing each material with *Pyrax* and Wisconsin talc both giving negative signs and with calcium carbonate and *AA Talc* giving positive signs. It has already been shown that a mixture of two materials of the opposite sign tend to neutralize the charge of each material and a mixture of materials of the same sign of charge tend to give increased charges.

With the exception of the flower sulphur samples, when the insecticides and fungicides given in table 1 were combined in proportions of one part to ten with *Pyrax* and Wisconsin talc, the original charges for *Pyrax* and Wisconsin talc samples were more or less reduced but the sign for all mixtures was negative. When combined in the same proportions with calcium carbonate and *AA Talc*, no reduction in charge was obtained, and in some cases the charges were greater than had been obtained with the natural samples of calcium carbonate and AA talc. The sign of charge in each case was (+). All samples of flowers of sulphur when mixed with the four test materials caused reductions in charge but the sign of charge for each material was unchanged.

The sign and the degree of charge for a number of finely ground plant and animal materials were also obtained and it was found that all such materials produced a (−) charge on the blower and a (+) charge in the dust cloud. The degree of charge varied from 500 volts for one sample of sabadilla to 8500 volts for a plant material known as *Baggo*. The charges for available derris and cube samples were approximately 4000 volts. However, the degree of charge for any material will be high or low depending upon the particle size. A series of lignin samples ground to different degrees of fineness were tested and a maximum charge of 6000 volts was obtained for a material passing through a 200-mesh screen. When the samples were ground to pass through a 325-mesh screen the charge was reduced and with superfine particles averaging less than 10 microns in size no charge was produced. A sample of dried pollen produced an average charge of 2500 volts. When powdered animal materials were tested, excreta

from clothes moths produced a charge of 2400 volts, samples of bone meal and dried blood each produced charges of 5000 volts.

A further effort was made to determine if the material in the delivery tube might have any effect on the sign of charge produced by any finely ground material. *Pyrax* producing a (−) sign and *AA Talc* producing a (+) sign were used as the principal test materials but some other mineral and plant materials were tested.

These materials were blown through tubes of copper, brass, lead, zinc, iron and aluminum. None of these materials seems to have any effect on the sign of charge.

Tests were also made to determine if pea plants might effect the sigh of charge. A wire leading to the voltmeter was attached to the base of a pea plant and the dust stream directed horizontally against the tip of the plant. If the surface of the stem was not broken no charge was recorded, but if the tip of the wire was pushed into the stem, inducted charges comparable to those recorded by direct contact with the dust tube and the voltmeter were observed. No lesions were noticed on the plant where contact with the dust stream was made. So far as observed the plant stem had no effect either on the sign or degree of charge for the materials tested. The same conditions prevailed when the plants were removed from the soil and the wire to the voltmeter inserted at the root end. No charge was obtained if the dust stream was directed so no dust particles came in contact with a part of the plant.

CONCLUSIONS.—A method has been devised for recording the electrostatic charges of blown dust with which consistent replicated readings are possible.

A preliminary study of samples of some commercial powdered insecticides and fungicides shows that there is a variation in the capacity of different mineral combinations to produce electrostatic charges. It is shown that the sign of charge for all the insecticides and fungicides studied is positive.

Some differences in dispersion apparently influenced by the degree of charge were also noticed although until a more complete study is made it cannot be said that they were significant from the standpoint of insect or plant disease control.

Finely ground plant and animal tissues of a granular nature produced electrostatic charges. Charges produced by plant materials were affected by the average particle size.

When powdered dusts producing electrostatic charges were blown through tubes made with different metals no noticeable change in the sign of the dust could be noted.

When dusts capable of developing electrostatic charges were blown against plants an induced charge opposite to that of the dust was imparted to the plant and carried for the full length of the plant.—4-10-44.

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The following revisions of or amendments to California quarantines have been made: (1) the European corn borer regulated area was extended, effective February 15, 1944, by the addition of 37 counties in Iowa, 1 in Minnesota, and 8 in Missouri; (2) oriental fruit moth area in California was extended May 22, 1944, to include portions of the California counties of Tulare, Fresno, and Kern; (3) the sweetpotato-weevil infested areas were modified to designate 140 Texas counties as free from restrictions, other than certification as to origin of restricted commodities; (4) the vetch weevil quarantine revision of February 10, 1944, added to the area under quarantine the States of Alabama, Connecticut, Idaho, Mississippi, and the District of Columbia, and set forth various approved treatments of seed for the weevil.

Toxicity of Cryolite to Mexican Bean Beetle Larvae

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One of the earliest recommended uses of cryolite was for the control of the Mexican bean beetle. Carter & Busbey (1939) list numerous papers on the use of cryolite for Mexican bean beetle control, but these have dealt mainly with field conditions. The present paper presents certain dosage-mortality and time-mortality data obtained under controlled laboratory conditions.

Table 1.—The mortality of Mexican bean beetle larvae instars feeding for 5 days on the same bean leaf sprayed with cryolite. One hundred larvae per test.

Lbs. in 100 Gals. Water	Mg. Deposit per Sq. Cm.	Larval Instar	Per Cent Mortality in Days				
			1	2	3	4	5
2 pounds	0.0106	1	14.1	39.8	66.0	83.9	96.6
		2	13.0	41.0	69.7	88.2	98.8
		3	4.0	12.0	35.4	86.5	97.9
		4	2.0	0.0	6.4	45.1	75.6
6 pounds	0.0313	1	11.1	39.8	74.5	97.8	100.0
		2	30.0	60.0	81.8	96.8	100.0
		3	12.0	32.0	61.6	92.7	100.0
		4	4.0	19.1	26.6	58.2	82.2
10 pounds	0.0560	1	29.3	64.3	84.1	100.0	100.0
		2	22.0	64.0	91.9	96.8	100.0
		3	23.0	58.0	83.8	95.7	98.9
		4	12.0	28.7	36.2	62.6	80.0
15 pounds	0.0796	1	22.0	60.2	87.2	96.8	100.0
		2	23.0	66.0	85.9	97.8	100.0
		3	23.0	62.0	84.8	92.7	97.9
		4	14.0	33.0	45.7	60.4	88.9
20 pounds	0.1002	1	42.4	77.6	94.7	98.9	100.0
		2	34.0	69.0	92.9	98.9	100.0
		3	24.0	66.0	83.8	95.7	100.0
		4	8.0	35.1	46.8	62.6	81.1

MATERIAL AND METHODS.—Natural cryolite (*Kryocide*, furnished by the Pennsylvania Salt Co.) was used in all tests. The material was micronized and was guaranteed to contain at least 90 per cent sodium fluoaluminate.

A belt sprayer adapted from one developed by Siegler & Munger (1935) was used in all tests. A bean leaf, approximately 40 sq. cm. in area was placed upside down in a petri dish on flannel. The dish and leaf was then passed twice through the spray cone on a moving belt, the spray being applied at 15 lbs. pressure per sq. inch. When the spray deposit had dried, the leaf was removed to a moist chamber until larvae were ready for exposure.

Tests were made in glass specimen dishes 112 mm. wide by 50 mm. deep. These dishes could be stacked and fitted together closely enough to prevent the escape of the larvae but not closely enough to make an air-tight seal. Moist filter paper, the size of the bottom of the dish, was used under the leaf. The hooked hairs on the upper surface of the bean leaf serve to keep the leaf closely appressed to the filter paper and prevent the larvae from crawling under the leaf and feeding on the unsprayed upper surface. Ten larvae were used in each dish. Experiments were performed at a constant temperature of 80 degrees F., and approximately 82 per cent relative humidity. Mortality records were made daily for 5 days.

Deposit determinations were made by weighing tared tin pill boxes of 40.7 sq. cm. area and were found to vary less than 6 per cent in the three deposits determined for each spray concentration.

In the first experiment where the susceptibility of the various instars to cryolite was being tested, the larvae fed for the 5-day duration of the experiment on the same sprayed leaves. In the second series of experiments the purpose was to determine how many days the larvae would have to feed in order to obtain a lethal dose. In these tests fresh sprayed leaves were supplied each day replacing those of the previous day. However, after periods of from 1 to 5 days in the various tests, clean unsprayed foliage was supplied in place of the sprayed leaves. As before, mortality data sere taken each day. Each figure in the tables has been corrected by Abbott's formula for the mortality in check tests.

DISCUSSION OF RESULTS.—In the first experiment not only instar, but also dosage, was varied. At given dosages, the younger larvae were most susceptible, and at given ages, those larvae feeding on leaves with the highest deposits died quickest. There was a greater difference in susceptibility between larvae of the third and fourth instar than between any two other age groups. This generalization seemed to apply at all deposit levels.

From the data in table 1, the 20 dosage-

mortality and 20 time-mortality curves were plotted according to the method of probits (Bliss 1935, 1937). Dosage mortality curves for the first, second and third instar larvae at the various times were generally straight lines with slight, but apparently random variation in angles of slope, commonly converging or crossing, and grouped close together. The dosage-mortality line for the fourth instar larvae was not closely associated with the group of lines representing the earlier instars, but indicated a markedly more resistant stage. The angle of slope for the fourth instar is generally parallel to that for the third instar. Figure 1 shows a typical group of dosage-mortality lines with mortality data after 3 days.

The same general conclusions can be drawn from a study of the 20 time-mortality curves. Figure 2 shows the time-mortality lines for the four instars at the 20-pound level. The most interesting observation made on these data is that in 19 cases out of 20, the time-mortality lines instead of being straight when rectified according to the method of probits, are curved upward. These consistently curved time-mortality lines are interpreted by the writers to indicate that cryolite kills

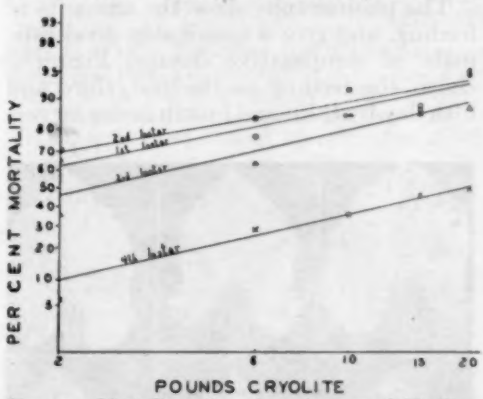

FIG. 1.—Mortality of various instars of Mexican bean beetle larvae after three days feeding on bean foliage sprayed with indicated amounts of cryolite.

bean beetle larvae in such a way that the relation between mortality and dosage is different from the relation between mortality and time. Put in other words, mortality is increased more by extended time of exposure to a given dosage, than by increasing the deposit a proportional amount. In all these discussions, it must be remembered that what is loosely called dosage is actually only deposit on foliage to which the larvae are exposed.

In order to study more closely the relations of time of feeding to mortality at various deposit levels, the experiments reported in tables 2 and 3 were performed.

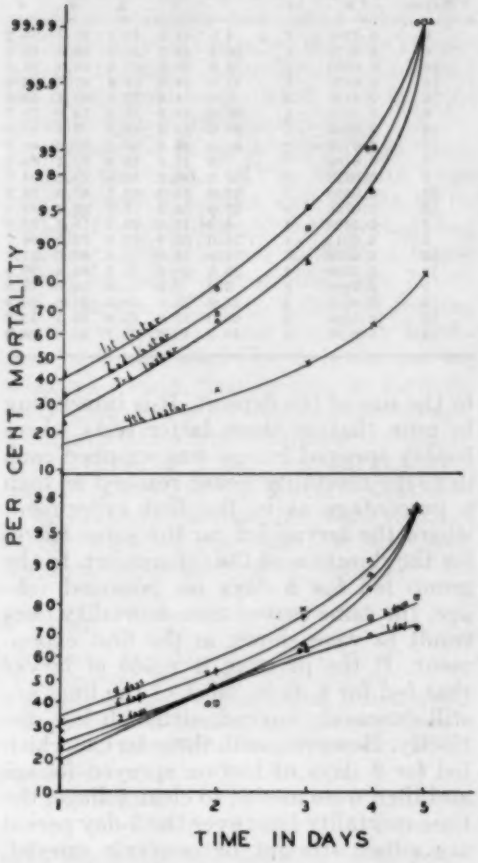

FIG. 2.—Above—Time-mortality curves for various instars of Mexican bean beetle larvae feeding on bean foliage sprayed with 20 lbs. cryolite per 100 gals.

Below—Mortality of third instar larvae of the Mexican bean beetle feeding from one to five days on bean foliage sprayed with 10 lbs. cryolite per 100 gals.

In these tests, poisoned foliage was removed after periods of from 1 to 5 days feeding.

A study of the data in tables 2 and 3 lead to the same conclusion drawn from the first experiment, namely: (1) The fourth instar is considerably more resistant to cryolite than the third, (2) increasing the deposit of cryolite increases the mortality, gradually, but not proportional

Table 2.—The mortality of third instar Mexican bean beetle larvae feeding 1, 2, 3, 4 and 5 days on bean leaves sprayed with various concentrations of cryolite. (One hundred larvae per test).

Lbs. in 100 Gals. of Water	Mg. Deposit 1 Sq. Cm.	Days of Feeding	Per Cent Mortality in Days				
			1	2	3	4	5
2	0.0106	1	4.0	13.0	26.2	31.3	37.2
6	0.0313	1	26.0	44.0	52.5	56.3	63.8
10	0.0560	1	19.0	39.0	63.6	69.8	73.4
15	0.0796	1	33.0	45.0	63.6	67.7	73.4
2	0.0106	2	2.0	13.0	27.3	38.5	50.0
6	0.0313	2	16.0	28.0	55.6	74.0	77.7
10	0.0560	2	23.0	39.0	63.6	77.1	83.0
15	0.0796	2	24.0	45.0	67.7	75.0	80.9
2	0.0106	3	7.0	11.0	43.4	77.1	89.4
6	0.0313	3	10.0	28.0	55.6	79.2	94.7
10	0.0560	3	25.0	44.0	65.7	87.5	96.8
15	0.0796	3	27.0	54.0	70.7	82.3	93.6
2	0.0106	4	4.0	11.0	33.3	78.1	95.7
6	0.0313	4	16.0	35.0	63.6	79.2	94.7
10	0.0560	4	31.0	52.0	77.8	90.6	95.7
15	0.0796	4	30.0	50.0	69.7	84.4	98.9
2	0.0106	5	3.0	9.0	31.3	56.3	93.6
6	0.0313	5	13.0	36.0	58.6	81.3	92.6
10	0.0560	5	29.0	52.0	75.8	84.4	95.7
15	0.0796	5	28.0	48.0	71.7	83.3	98.9

Table 3.—The mortality of fourth instar Mexican bean beetle larvae feeding 1, 2, 3, 4, and 5 days on bean leaves sprayed with various concentrations of cryolite. (Seventy-four to 80 larvae per test.)

Lbs. in 100 Gals. of Water	Mg. Deposit 1 Sq. Cm.	Days of Feeding	Per Cent Mortality in Days				
			1	2	3	4	5
2	0.0106	1	1.3	6.3	7.6	8.9	16.7
6	0.0313	1	5.0	21.3	25.0	22.4	20.8
10	0.0560	1	15.0	26.3	35.0	39.4	45.2
15	0.0796	1	14.9	23.5	43.2	44.6	48.1
2	0.0106	2	0.0	5.0	7.5	13.2	25.2
6	0.0313	2	15.2	24.1	36.8	45.4	48.9
10	0.0560	2	10.0	32.5	43.8	46.1	56.7
15	0.0796	2	20.0	38.7	42.7	48.1	46.1
2	0.0106	3	1.3	5.0	15.0	30.3	45.2
6	0.0313	3	7.6	25.3	34.2	61.4	73.7
10	0.0560	3	10.0	35.0	47.5	63.2	74.4
15	0.0796	3	23.8	53.8	65.0	78.9	88.5
2	0.0106	4	0.0	6.4	16.3	39.4	65.4
6	0.0313	4	5.0	17.5	40.0	59.3	74.1
10	0.0560	4	18.8	32.5	45.0	67.2	77.5
15	0.0796	4	18.8	43.3	50.0	68.4	87.1
2	0.0106	5	1.3	2.8	8.8	38.2	65.2
6	0.0313	5	5.0	21.3	41.3	60.5	74.1
10	0.0560	5	16.5	35.4	48.1	68.0	85.4
15	0.0796	5	21.3	46.3	52.5	80.3	90.0

to the size of the deposit. It is interesting to note that in these latter tests where freshly sprayed foliage was supplied each day, the mortality never reached as high a percentage as in the first experiment where the larvae fed on the same leaves for the duration of the experiment. In the group fed for 5 days on poisoned foliage, the same curved time-mortality lines result as were shown in the first experiment. If the plotting is made of larvae that fed for 4 days, most of the lines are still concavely curved, although less distinctly. However, with those larvae which fed for 3 days or less on sprayed foliage and then were moved to clean foliage, the time-mortality lines over the 5-day period are either straight or convexly curved. Such a group of curves are shown in figure 2 representing the mortality during the 5 days of the experiment of third instar larvae feeding from 1 to 5 days on a deposit of 0.560 mg./cryolite per sq. cm. When these curves are studied, they indicate that the concavely curved time-mortality lines result from the nature of the feeding of the larvae on cryolite deposits, and not the nature of the toxic action of the insecticide after it is ingested.

These experiments further serve to emphasize that cryolite is a slow-acting insecticide. Fourth instar larvae fed for one day were less than half dead after 5 days. Evidently fourth instar larvae must feed for 3 or 4 days even with heavy deposits in order to obtain a high mortality. With third instar larvae 2 days' feeding with the higher doses seem to be necessary. On the evidence of these laboratory tests, it would seem wise to emphasize in field recommendations, that in order to obtain good results with cryolite the applications should be made before the larvae have reached the fourth instar.

The photographs show the amounts of feeding, and give a reasonably good estimate of comparative dosage. Figure 3 shows the feeding on the first, third and fifth day by third and fourth instar larvae.

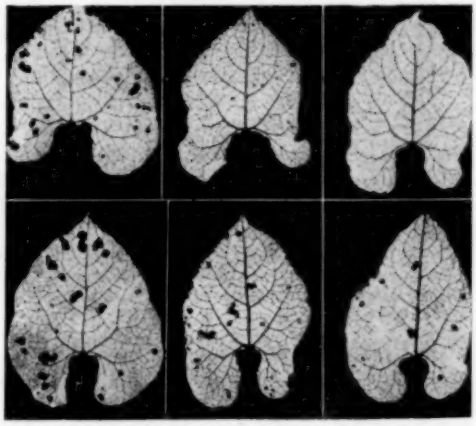

FIG. 3.—Above—Feeding on first, third and fifth day by 10 third instar larvae on bean foliage sprayed with cryolite at 10 lbs. to 100 gals.
Below—Feeding on first, third and fifth day by 10 fourth instar larvae on bean foliage sprayed with cryolite at 10 lbs., to 100 gals.

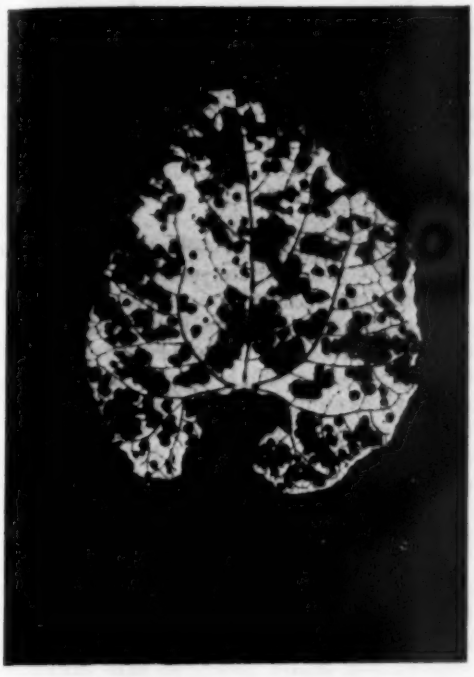

FIG. 4.—After 1 day of feeding by 10 third instar larvae which had previously fed 2 days on leaves sprayed with 10 lbs. cryolite per 100 gals.

Leaves freshly sprayed with cyrolite at the rate of 10 lbs. to 100 gals, were supplied each day. It is evident from these photographs, that while the older larvae will feed each day for at least 5 days, the third instar larvae obtain a lethal dose around the second day, since their feeding has almost entirely ceased by the third day. The fact that cryolite deposits are truly repellent is shown in figure 4 which is a photograph of a leaf after one day's feeding by 10 third instar larvae which had fed on sprayed leaves for 2 days prior to placing them on the clean foliage.

SUMMARY.—In laboratory cage tests against the larvae of the Mexican bean beetle, cryolite sprays were shown to be highly toxic, although slow acting and repellent. First, second and third instar larvae are more susceptible than fourth instar larvae. Three to 4 days of feeding on heavy deposits seem necessary to obtain high kills with fourth instar larvae, whereas, 2 days, feeding give the same results with larvae of the younger stages. Time-mortality lines plotted according to the methods of probits are convex curves instead of straight lines and are considered to be shaped by the nature of the feeding, rather than by the nature of the toxic action of ingested cryolite.—7-5-44.

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Notices of recent revisions of Arizona State quarantines carry an introductory note briefly stating the nature of the changes made by such revisions. This time-saving practice, which is also employed in the issuance of California notices, is a great convenience in saving the reader the necessity of checking back with former quarantines to determine the purpose and extent of the respective revisions. These changes in the Arizona regulations and quarantines, as adopted in April or May 1944 included: (1) lifting all tolerances as to entry of crown-gall infected plants; (2) under the general citrus quarantine, providing for entry of citrus nursery stock from any part of California into any part of Arizona, under permit; (3) providing for fumigation as a condition of entry of cured sweetpotatoes and so-called yams from sweetpotato weevil regulated areas, and redesignating such areas by removing Conecuh and Crenshaw Counties, Ala., Charlton County, Ga., and 9 Mississippi counties, and by the addition of Chatham County, Ga., De Soto and Sabine Parishes, La., Greene County, Miss., and Shelby County, Tex.

A revision of Washington State quarantine relating to the greater bulb fly and the bulb nematode, which became effective March 15, 1944, broadens the provisions with respect to treatment of narcissus bulbs for these pests, and requires appropriate labeling of containers of certified narcissus bulbs taken from original containers for reshipment in small lots. The Washington quarantine pertaining to vetch weevil, as revised effective April 13, 1944, extends the regulated areas to apply to 13 States and the District of Columbia and provides for acceptance of vetch seed from such areas when treated under one of the various approved schedules, or when originating in noninfested area.

Heliothis virescens as a Pest of Cotton, with Notes on Host Plants in Peru

EDSON J. HAMBLETON,[1] *U.S.D.A., Office of Foreign Agricultural Relations*

The tobacco budworm has long been known as a serious pest throughout the tobacco-growing sections of Florida, Georgia, Alabama, and Louisiana, but only within the past 10 years have observations of this insect shown its increasing importance as a pest of cotton. Folsom (1936) reports that *Heliothis virescens* was almost as prevalent on cotton as (F.) *H. armigera* (Hbn.) during the 1934 season in the vicinity of Tallulah, La. This seems to be the first published record of *virescens* as a cotton pest in continental United States. He states that "probably much of the damage attributed to *obsoleta* is done by *virescens*" and was of the opinion that the species had been overlooked on cotton because of its resemblance to the congeneric H. *obsoleta*, the bollworm.

HISTORICAL BACKGROUND.—The first record of the tobacco budworm as a pest of cotton was made, however, by Wilson (1923) in the Virgin Isles. *Heliothis virescens* was found there with *obsoleta*, but only a few larvae of the former were taken from cotton bolls. He mentioned, however, that the budworm was present in some numbers in okra pods and tobacco seed pods. Nothing more appeared in the literature concerning the status of *H. virescens* as a cotton pest until Townsend (1928) discovered it on cotton plants in Peru, where it was not causing any noticeable damage. Later Pope (1929) reported the species on cotton from northern Peru, and no further mention of the insect in that country appeared until 10 years afterward.

Until 1935, practically nothing was known of *Heliothis virescens* in Brazil. Hambleton (1935) recorded the species attracted to lights at Viçosa, Minas Geraes. Hambleton & Forbes (1935) first recorded *virescens* from cotton in the State of São Paulo. Local outbreaks occurred during 1935, causing some damage in the northwestern part of the State. A more complete account of this outbreak was given by Mendes (1937), who included biological data on the species and described the nature of its injury to cotton in São Paulo.

Since, as a pest of tobacco, *Heliothis virescens* is well known, particularly in the United States and Cuba, the fact that its occurrence on cotton has been known during the past two decades only would seem to indicate that (1) the food habits of the species have changed, (2) the species has been confused with *H. obsoleta* in the field, or (3) a distinct biological race has been developed. *H. armigera* is perhaps more widely distributed than *H. virescens*, and its host plants are likely more numerous. The two species in immature stages are quite similar; and, since *H. armigera* is the better known of the two, a reasonable supposition is that both have been considered for many years as one and the same species by field workers. This supposition is being accepted because the ecological conditions are so diverse throughout the wide range of its distribution that they alone could scarcely be held responsible for a change in its food habits if this were considered the reason for not finding *H. virescens* on cotton at an earlier date. Variation in host preferences is distinct for the two species in the same, or in widely separated areas. For example, in the West Indies Wolcott (1933) found *H. virescens* more common on pigeonpea, *Cajanus indicus*, but it was sometimes noted on cotton. He says: "Cuba is the only tobacco-producing island of the West Indies in which *H. virescens* occurs as a pest in tobacco fields. It appears to be present in all the other islands and at times in considerable abundance, yet it does not attack tobacco . . . In Jamaica, *obsoleta*, which rarely attacks tobacco elsewhere, is reported as being the typical budworm of tobacco." Bruner and Scaramuzza (1938) also report that of all the Antilles *H. virescens* is a pest of tobacco in Cuba alone, that it is rare on the other islands, and not to be found in Jamaica.

The status of *Heliothis obsoleta* in Puerto Rico must be similar to that of this species in Peru. Fife (1939) did not report that this insect attacked cotton on

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the island, but he did observe *H. virescens* on this host and remarked that "but rarely, if ever, does it become a pest there."

In Argentina, Hayward (1942) lists *Heliothis armigera* as the species attacking cotton in the vicinity of Tucuman. The form *H. distincta* Schaus of *H. virescens* also occurs in the same locality and is reported on tobacco, tomato, and flax, but cotton is not mentioned as a host.

As yet there is no evidence to justify the hypothesis that a distinct biological race of *H. virescens* has been developed as a possible explanation for its comparatively recent occurrence as a destructive cotton pest. Perhaps a better interpretation of the fact could be gained from studies made in localities where the insect is a pest of cotton, in order to determine its food plants other than cotton, the ecology of the areas where cotton is cultivated, and the part natural enemies play in effecting changes in its population over a period of years.

CONDITIONS IN BRAZIL AND PERU.—The occurrence of the tobacco budworm as a pest of cotton in Brazil and Peru at approximately the same time can only be interpreted as a mere coincidence. The probabilities, too, are that Wille (1940) misinterpreted the facts relative to the situation of *Heliothis virescens* as a potential cotton pest and the nature of the crop in the two Republics. He was also under the impression that the insect was unknown as an enemy of cotton except in South America and the Virgin Isles.

Heliothis virescens is not considered a pest of much consequence in Brazil. In the São Paulo area, where local outbreaks occurred in 1935, cotton was a comparatively new crop. In fact, in one of the two areas where the insect appeared, the crop had been planted for the first time in virgin forest land that had been cleared the previous year. From 1935 to 1939, no reoccurence of *H. virescens* was observed in that locality. In the other area mentioned by Mendes cotton cultivation had been in progress but a few years, and much new land was being prepared for increasing the cotton acreage. Any conclusion regarding the immediate future of the pest at that time would have been premature, for both the crop and the insect damage were new in the area. Incidentally, *virescens* has not been found in any of the older cotton-growing areas of the country. An interesting point is that the cotton bollworm, *H. armigera* is commonly found attacking corn and other minor crops throughout much of Brazil, but the damage it does is not nearly so great as that caused by the same species to corn and other crops in the United States. It has been observed on cotton in Brazil, but only on rare occasions. During 5 years in the State of São Paulo, the writer failed to discover a single specimen of *armigera* on cotton.

A reasonable assumption is that natural enemies have been important factors in maintaining low populations of both *armigera* and *virescens* in the cotton fields of Brazil. And yet the fact cannot be discounted that conditions surrounding the present-day cotton crop in São Paulo are the result of a changed agriculture developed largely within the past 10 or 15 years. The crop as a whole has been successful, but surely in so short a period all insect pests, as well as their enemies, have not had time to become firmly established or adjusted to their changed habitat. Both ecological and climatic conditions are known, however, to favor the propagation of many of the enemies of cotton pests. In this connection, the predaceous Hemiptera and the parasitic Diptera have undoubtedly held the species of *Heliothis* well in check. *Orius insidiosus* (Say) is extremely abundant on both cotton and corn. Its effectiveness in destroying the eggs and larvae of *H. armigera* in the United States has been fully discussed by Barber (1936). Perhaps the work of this predator, and others, may help to explain the relative unimportance of both *H. armigera* and *H. virescens* as cotton pests in Brazil.

From 1939 to 1943, the tobacco budworm ranked as the most destructive insect enemy of the cotton plant in the Cañete Valley of Peru. A series of reports relative to the insect in this valley was published by Maria (1939). This included general information on the insect and measures to be taken relative to its control. Wille traced the history of *Heliothis virescens* as a cotton pest in Peru, its development as a serious menace to the crop since 1937, and the various means employed to combat the insect. In a series of monthly reports to the Sociedad Nacional Agraria, Hambleton (1940–43)

made additional comments while studying the biology and control of *H. virescens* in the Cañete Valley.

The sequence of events leading up to the time when *Heliothis virescens* became a threat to the cotton crop in the Cañete Valley and its status as a pest until 1943 is, without doubt, unique in character. This particular cotton bollworm, or tobacco budworm as it is known in the United States, is present in most of the cotton-growing valleys along the narrow coastal strip extending from northern Peru to the Ica Valley in the south. Nevertheless, it was little known in any of these valleys and had always been considered relatively unimportant. The Cañete Valley, on the other hand, was originally planted during many years largely to sugarcane, with some cotton, corn, and minor crops. Cotton, however, gradually replaced sugarcane as a major crop, and by 1934 the latter industry disappeared entirely from the valley. From 1920 to 1936 both the acreage and yields of cotton showed a steady increase, and no appreciable loss from insect pests was registered, except that caused by the leafworm *Anomis texana* Riley. From a record production in 1936, the annual yield in the Cañete Valley had decreased in the year 1940 by approximately 55,000 quintales,[1] largely on account of *Heliothis virescens*. This was also accompanied by a severe infestation of *Aphis gossypii* Glover, resulting from heavy arsenical-dust applications and further aggravated by improper irrigation practices. This situation was most alarming, since it resulted in an average loss of from 30 to 40 per cent of the crop, and an extraordinary expense was incurred in attempting to control the pests with insecticides. The status of the cotton aphid in relation to arsenical dusting of cotton in the Cañete Valley has been comparable to similar developments in certain areas of the Cotton Belt in the United States. Likewise, the factors influencing populations of *H. virescens* in Peru have undoubtedly paralleled results obtained on *H. armigera* in the United States during recent years. Results obtained by Dunnam & Clarke (1941). Smith & Fontenot (1942), and Ewing & Ivy (1943) are characteristic of developments in Peru.

Roughly some 60,000 acres are under cultivation in the Cañete Valley. With modern agricultural equipment and an abundant water supply in most of the valley, cotton has until recently occupied most of the available area in cultivation. The mean temperature during the long growing season from September to May averages about 70° F. For the cultivation of long-staple Tangüis cotton, which requires a much longer growing season than the short-staple varieties, climatic conditions there are quite satisfactory. A long growing season, with suitable moisture requirements and ideal temperatures, also affords insect pests like *Heliothis* a favorable environment in which they may propagate throughout the entire year. Undoubtedly *H. virescens* has long been present in the valley, and as cotton slowly gained dominance by replacement of other crops, this species gradually built up to alarming numbers and became a potential menace to cotton production in the valley.

Three years of intensive effort to remedy the situation by means of various arsenical dusts and other well-known insecticides proved only costly and ineffective against the larvae of *Heliothis virescens*. Egg deposition began on cotton seedlings and on ratoon cotton in October and November. Deposition steadily increased as moths emerged from hibernation over a period of from 8 to 10 weeks. Infestation developed earlier in the season in the ratoon cotton, and as soon as this was no longer attractive to egg-laying moths their attention was focused on the newly planted crop. At the peak of infestation the cotton plant had usually attained such a height that it became practically impossible to use dusting equipment from the ground. Where dusting was done by airplane at regular intervals, the outcome in the end was equally disastrous. Experimental plots dusted with arsenicals every 7 days for a total of six applications produced less cotton than untreated checks. There was no evidence in many fields under observation to show that bollworm infestation was reduced to any appreciable extent with arsenical dusts. Large-scale dusting operations repeated every 2 or 3 weeks during the height of infestation were futile, and the resulting effects on aphid increase developed into such a complex each season that the need for seeking other means of relief became most urgent.

[1] 1 quintal equals about 100 pounds.

The control of the cotton aphid, *Aphis gossypii* Glover, in the *Heliothis*-infested fields became every bit as complicated as the bollworm problem itself. Heavy infestations built up rapidly and, where uncontrolled, resulted in much shedding of squares, bolls, and foliage.

Cotton cultivation in the Cañete Valley had become so intensified that conditions were favorable for the development of the bollworm but unfavorable for its natural enemies. Various species of parasites and predators which had been under observation were unable to multiply sufficiently to become effective agents of natural control. Population studies revealed that some species multiplied toward the end oft he growing season but were never present in sufficient numbers to prevent the the usual amount of damage from occurring to the succeeding cotton crop. Undoubtedly the development of natural enemies during the winter months was reduced to a minimum, since there was little vegetation, and few secondary hosts to provide shelter or food.

The utilization of the chickpea or garbanzo plant, *Cicer arietinum*, reported by Wille as a successful trap crop for bollworms, was discontinued entirely after one season's (1938–39) experience, because it was found impracticable as well as ineffective in reducing *virescens* infestation in cotton.

With the introduction of flax as a winter crop and with other crops being planted on a larger scale than previously, by 1940 the ecological status of the valley was subjected to changes that were eventually to modify the entire insect situation with respect to *Heliothis virescens*. Fields of flax were often followed with summer crops of corn, beans, sweet potatoes, etc., or, if left idle soon became overrun with weeds. This succession of crops provided more suitable conditions for an increase of natural enemies. The cultivation of corn was especially encouraged, because it served as a host to many insect species upon which such predators as *Nabis punctipennis* Blanch. and *Paratriphleps laeviusculus* Champ. found ideal conditions to propagate. These two beneficial species were earlier found to prey upon first- and second-instar larvae and eggs of *Heliothis*, respectively. Their population gradually developed under the changed conditions as they found other hosts to prey upon after the cotton crop had terminated. Of the two predators, *Nabis punctipennis* proved the more valuable.

In more or less the same manner, parasitization by two or more tachinid flies of the genus *Archytas*, became noticeable. Flax cutworms, chiefly *Agrotis ypsilon* (Rott.), *Euxoa bilitura* (Guen.) and *Laphygma frugiperda* (S. & A.) at times were quite serious during the winter season from June to August, when there was no cotton. These flies readily found access to additional host material and were able to produce another generation or two by the time *Heliothis* again became active on cotton. Unlike the predators, which destroy either eggs or small larvae before much destruction to plant tissue occurs, the larvae of the tachinid fly feeds internally on its host until the latter reaches maturity as a full-grown larva. Pasasitism of this nature aids chiefly in reducing the number of moths that are responsible for the first generation of larvae in the following cotton crop.

Losses from *Heliothis* and aphid injury to cotton were extremely heavy during the first 2 years that flax was sown on a large scale. Egg counts in this period reached a maximum average of nearly 400 eggs per 100 cotton terminals in some fields. Experimental evidence during this same period demonstrated quite clearly that *H. virescens* could not be controlled on cotton with the insecticides that were then in current use. On the basis of this conclusion, and taking into consideration the possibility of natural control playing a more prominent rôle, a general recommendation was made for all growers not to dust their cotton for *H. virescens* during the 1942–43 growing season.

Until December 1942, bollworm activity was more or less comparable with that of previous years. Terminal injury was noticeable in ratoon cotton, and egg counts had steadily increased to an average of 45 per 100 terminal buds. Following this period egg deposition decreased rapidly, and as the season progressed infestation became of less importance throughout the valley. Cotton planted that season was practically free from *H. virescens* for the first time since the insect had become so destructive. Field counts and observations indicated that the first-generation larvae had practically been annihilated by adults and nymphs of *Nabis*

punctipennis. Counts of these predators indicated as many as one individual for every two terminal growths in some cotton fields. When first- and second-instar bollworm larvae migrated from within the enclosed terminal buds, these predators were seen to prey upon them on various occasions. Difficulty was experienced in finding fourth- or fifth-instar larvae in fields where nabids were plentiful. Cotton grew normally, produced well, and was not dusted with arsenicals until late in the season for leafworms.

The decline in *Heliothis* infestation was attributed primarily to increased activity of its natural enemies. A more balanced type of agriculture, including some rotation of new crops, the planting of corn within cotton fields or adjacent to them, and use of less water in irrigation, all aided in restoring conditions favorable for the propagation of natural enemies and the ultimate decline of *Heliothis*. Cultural control methods thus proved superior and far more economical than chemical control under Cañete conditions. That *Heliothis virescens* would again constitute a menace in the valley does not seem likely unless growers resort to agricultural practices similar to those that are believed to have been largely responsible for the outbreak in the first place. In this connection, an interesting point is that the tobacco budworm is of practically no importance in most valleys of Perú where a diversified type of agriculture is maintained.

HOST PLANTS OF *Heliothis virescens* IN PERU.—Climatic conditions in the cotton-growing areas of Peru are conducive to some *Heliothis* activity during the entire year. In the Cañete Valley, activity is markedly reduced as cool weather approaches, and *H. virescens* is largely in a state of hibernation in the pupal stage. Nevertheless, active larvae may be found to some extent on a number of secondary host plants throughout the year. None of these, however, exists in such quantity as to be of importance in building up a sufficiently heavy population of *H. virescens* to be of much consequence to the cotton crop. Even though both eggs and larvae have been observed during all the winter months, reproduction and growth are greatly retarded. From the nature of the food plants attractive to *H. virescens* during this season, none provide so much protection as the cotton plant. Consequently, larvae are usually exposed and subject to attack by natural enemies. The following notes concern those plants upon which *H. virescens* has been observed in Peru. Unless otherwise stated, the records are all from the Cañete Valley.

Flax.—Observations on this crop during three seasons revealed that the blossom buds and seed capsules are occasionally attacked, but the damage is insignificant. This is to be expected inasmuch as flax is a winter crop and *Heliothis* is only partially active during that season.

Tomato.—In September 1942, *virescens* moths from overwintering pupae emerged in greater numbers than in previous years. Of several new hosts recorded, tomato plants were found to have attracted moths for egg deposition. Larvae hatched from eggs taken on this host, but they were unable to develop beyond the second instar. No damage to fruits was observed.

Beans.—Eggs and very small larvae were collected on ordinary field beans in April and May 1942. No further observation was made, and no damage to this crop by *H. virescens* was ever noted.

Chickpea.—Ordinarily this plant is quite attractive to budworms, but it is not extensively cultivated in the valley. When used as a possible trap crop against *Heliothis virescens*, it is reported to have been heavily infested in 1939.

Pigeonpea.—The tobacco budworm is practically unknown to cotton growers in the Piura and Chira Valleys, since it is of rare occurrence on cotton there. The pigeonpea, *Cajanus indicus*, is grown on a small scale by the natives as a perennial for its seed. In July 1941, many of the buds and seed pods of this plant were found infested with *H. virescens* larvae in the Piura Valley. Both valleys possess a larger variety of native plants and insects than most of the other cotton-growing valleys of Peru. Several species of Anthocoridae are commonly found associated with cotton, especially in the Chira Valley *H. virescens*, under conditions found there, is not likely ever to become of significance as a pest of cotton.

Hyacinth bean.—The buds, blossoms, and seed pods of *Dolichos lablab*, or the "lenteja chilena" as it is known in Peru, are quite attractive to *Heliothis virescens* during the winter months, when the plant makes its most profuse growth. Although

these beans are not cultivated extensively, small plantings are frequently found infested, often to such extent that practically no crop is produced.

Squash.—On one occasion a small planting alongside a cotton field was found, with nearly full-grown larvae of *Heliothis virescens* attacking the blossom buds and young squashes. This infestation damaged most of the crop.

Sage.—In a small experimental planting of *Salvia officinalis* one half-grown larva of *Heliothis virescens* was found feeding on the foliage of this plant in March 1942.

Acalypha infesta.—This species of Euphorbiaceae was found growing as a weed in a planting of cowpeas. In November 1942, larvae of *Heliothis virescens* were observed feeding on the blossoms and clustered terminal growths of this plant.

Nicotiana paniculata.—During the winter months an occasional example of this plant makes its appearance in cultivated fields. Blossom buds containing eggs and first-instar larvae of *Heliothis virescens* were found on several plants in September 1942.

Nicandra physaloides.—Several small green fruits of this common solanaceous plant, containing larvae of *Heliothis virescens*, were received from the Chincha Valley in August 1941.

Geranium spp.—Several varieties of geraniums are frequently injured by virescens larvae. Moths are attracted to these plants in late winter and deposit large quantities of eggs on the blossom clusters. Larvae often destroy the entire cluster and plants fail to produce flowers. A large double-flowered red variety was observed to be immune to the attack of this insect. Injury to geraniums has also been observed in Miraflores, Lima.

Rosa sp.—Eggs of *Heliothis virescens* were observed on the buds of a large white tea rose for the first time in September 1941. Later it was revealed that a number of these buds had been injured by larvae boring through the petals before they had unrolled. Several flowers were found containing larvae nearly two-thirds grown. Red roses of a similar variety in the same plots were not attacked.

Callendula officinalis.—Budworm larvae feed on the buds and open blossoms of this ornamental from August to January in Cañete and Lima.

Helichrysum bracteatum.—Injury to the common strawflower occurs from September to January. Larvae bore into the terminal growths, often killing these portions of the plant.

Antirrhinum majus.—Larvae of *Heliothis virescens* frequently injure the buds, flowers, and seeds pods of snapdragons during the winter months in Cañete and Lima.

Lathyrus odoratus.—One small planting under observation in November was found with numerous eggs of *Heliothis virescens*. Only slight injury to the blossoms resulted.

Petunia, *Verbena*, *Ageratum*, and an undetermined composite, all common garden varieties, were also found partially injured by the larvae of *Heliothis virescens*, during November 1942.—6-27-44.

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Comparative Toxicities of Copper Hydroarsenate and Copper Hydroarsenate-Arsenite Mixtures.[1]

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The purpose of this investigation was to evaluate as stomach poisons two new products containing basic copper arsenicals in comparison with certain standard arsenicals.

The literature on basic copper arsenicals is not large; and publications in which data from quantitative laboratory experiments are presented are few in number. Witman *et al.* (1939) published the chemical and physical properties of a basic copper arsenate, made under rigid laboratory conditions, and Waters *et al.* (1939) investigated its insecticidal properties. This basic copper arsenate acted more slowly on southern armyworms, *Prodenia eridania* (Cram.), than lead arsenate, even though the median lethal dose (LD50) of the copper compound lay between 0.18 and 0.20 milligram per gram of body weight, while the dose of lead arsenate fell between 0.25 and 0.28. Ellisor & Blair (1940) found the LD50s of lead arsenate, calcium arsenate, and basic copper arsenate for the southern armyworm at 80° F. to be 0.14, 0.16 and 0.13 mg./mg. of body weight respectively. Against the velvetbean caterpillar, *Anticarsia gemmatilis* (Hbn.), at 80° F., the LD50s of the same compounds were 0.11, 0.10, and 0.14 mg./mg. of body weight respectively. The median lethal doses in experiments at 60° F. ranged somewhat lower.

MATERIALS.—Copper hydroarsenate and copper hydroarsenate-arsenite products were the new materials under investigation (Seibert & Roller 1943). Two special arsenicals, paris green-calcium arsenate (a reacted mixture of paris green and calcium arsenate containing cuprous compounds)[4], and cupric metaarsenite[5] were included in certain experiments. The standard arsenicals used in all tests were acid lead arsenate, calcium arsenate and paris green. Specifications of the materials are given below:

Copper-containing Arsenicals
Copper hydroarsenate precipitated on calcium sulfate, and containing
Basic cupric arsenate $[Cu_3(AsO_4)_2 \cdot Cu(OH)_2]$[6] not less than 40%.

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[4] *Calgreen.* [5] *Air-Flo Green.*
[6] Also may be expressed as $Cu(CuOH)AsO_4$.

Inert ingredients not more than 60%.
As_2O_5 19.5%.
Copper, metallic, 20.3%.
Water-soluble arsenic (As_2O_5) 0.81% (pH 8).
Copper hydroarsenate-arsenite precipitated on calcium sulfate and containing
Basic cupric arsenate approximately 32%.
Basic cupric arsenite $[Cu_3(AsO_3)_2 \cdot Cu(OH)_2]$[1] approximately 8%.
Inert ingredients not more than 60%.
As_2O_5 13.47%.
As_2O_3 3.81%.
Copper, metallic 18.85%.
Water-soluble arsenic (As_2O_5) 0.60% (pH 8.2).
Cupric metaarsenite $Cu(AsO_3)_2 \cdot H_2O$ containing
As_2O_3 70.16%.
Water-soluble arsenic (As_2O_3) 0.57%.
Paris green-calcium arsenate reaction product[2] containing
As_2O_5 39.42%.
As_2O_3 4.63%.
Water-soluble arsenic (As_2O_5) 0.26%.
Paris green containing
As_2O_3 55.23%.
Water-soluble arsenic (As_2O_3) 1.72%.

Non-copper Arsenicals
Acid lead arsenate containing
As_2O_5 32.72%.
Water-soluble arsenic (As_2O_5) 0.06%.
Calcium arsenate, commercial, containing
As_2O_5 41.80%.
Water-soluble arsenic (As_2O_5) 0.80%.

The test insects included the following species:

Colorado potato beetle, *Leptinotarsa decemlineata* (Say): Laboratory reared fourth instar larvae.

Imported cabbage worm, *Pieris rapae* (L.). Laboratory reared fifth instar larvae.

Corn earworm, *Heliothis armigera* (Hbn.). Practically all were laboratory reared fifth instar larvae. A few field collected larvae were used in the first tests.

Differential grasshopper, *Melanoplus differentialis* (Thomas). Field collected adults.

Spotted cucumber beetle, *Diabrotica duodecimpunctata* (Fab.). Field collected adults.

METHODS AND RESULTS: *Colorado potato beetle.*—To administer measured doses of poison to the larvae, a single-leaf method (Ellisor & Boyd 1938), was employed. Squares of potato foliage, 400 square millimeters in area, were painted on one side with a five per cent starch paste. Twenty of these squares were placed around two tin plates (4000 square millimeters in area) which were located on the sliding drawer of a dusting table designed after that of Swingle *et al.* (1941). The dust was puffed from the metal cup in the top of the dutings tower at an air pressure of ten pounds per square inch. Ten seconds were allowed for the coarse particles to settle from the dust cloud onto the sliding drawer. Then the section bearing the plates and leaf squares was pushed under the tower and held there for one minute to allow the dust to settle on the leaf squares and tin plates. The dust deposit per square millimeter which fell on the leaf squares was calculated from the weight of dust on the tin plates.

The leaf squares were allowed to dry before they were presented to the larvae. A pin, which had a small square of celluloid near its head, was thrust through an inverted dried leaf square, and also through a square of heavy paper immediately beneath the leaf. The leaf square was adjusted one eight of an inch above the paper square, while the celluloid square rested upon the leaf. Then the pin was pushed into moist sand in a feeding jar. A weighed larva was placed in the jar and allowed to consume a certain amount of leaf. Usually the feeding time did not exceed ten or fifteen minutes, but with the more repellent compounds the larva was permitted to feed as long as twenty-four hours. The area eaten was measured under a binocular microscope by placing the leaf square over a millimeter-ruled scale. Each larva was retained for three days to note results. Untreated leaves and starch-coated leaves served as controls in each set of twenty larvae. Mortality in the controls was not appreciable. The experiments were made at room temperature and the prevailing air humidity.

The Colorado potato beetle larvae used in these experiments averaged 0.075 gram in weight. The poison ingested by each larva, was converted to milligrams per gram of larva body weight. The data were grouped according to the amount of poison ingested per unit of body weight, and the percentage of mortality was calculated for each group. Accepting the thesis that the LD50 is the best point of comparison between toxicity data, the grouped data were analyzed by the Bliss dosage-mortality method (1935). In table 1 are presented the LD50s with their 5 per cent limits, the slopes of the regression lines with their standard errors, and the chi-square values for variability with their probabilities.

Copper hydroarsenate and copper hydroarsenate-arsenite proved quite repellent and attempts to force the larvae to eat resulted in little feeding.

Imported Cabbage Worm.—The single-leaf method was used also with imported

[1] Theoretical formula. See Carter *et al.* (1943) for a recent discussion of the arsenates of copper.
[2] *Calgreen.*

Table 1.—Toxicity of seven arsenicals to fourth-instar larvae of the Colorado potato beetle.

Material	No. Insects	LD50±5% Limits (Mg./gm. Body Wgt.)	Slope± Standard Error	Chi-Square	P
Copper hydroarsenate	266	0.13 ±.019	2.92±.49	1.23	.73
Copper hydroarsenate-arsenite	496	0.25[1]	1.78±.36	0.52	.89
Lead arsenate	234	0.073±.011	2.87±.42	6.30	.046
Calcium arsenate	178	0.052±.008	2.88±.54	0.08	.99
Paris green	194	0.035±.015	1.02±.47	5.44	.15
Paris green-calcium arsenate	131	0.051±.010	2.96±.60	2.73	.44
Cupric metaarsenite	98	0.040±.008	3.25±.72	0.01	.99

[1] Value determined by extrapolation.

cabbage worms of 0.074 gram average weight. The only change in the method was the incorporation of 0.5 per cent blood albumin in the water before the 5 per cent starch paste was made. This was necessary to facilitate the spreading of the paste over the waxy cabbage leaf squares. The albumin-starch checks, like the untreated checks, showed no significant mortality.

The paris green-calcium arsenate product and cupric metaarsenite were not tested against this insect. Repeated trials with calcium arsenate failed to indicate definite intermediate or toxic zones. Although fifty larvae ingested more than 1.0 milligram of calcium arsenate per gram of body weight, only eleven of them died. The Bliss (1938) method of computing regression lines from small samples was used in the analysis of the data summarized in table 2.

Corn Earworm.—Leaf squares of cranberry beans were used to administer the poisons to this insect. The average larval weight was 0.315 gram. Copper hydroarsenate and copper hydroarsenate-arsenite were very repellent to the last instar larvae. Very small quantities of treated foliage were consumed even during a period of twenty-four hours. The doses of copper hydroarsenate fed to one hundred and forty-two larvae varied from 0.007 to 0.568 mg./gm. body weight. Nine individuals, scattered over the range, succumbed. However, the eleven larvae consuming the highest dosages, from 0.360 to 0.568 mg./gm. body weight, did not die in seventy-two hours. One hundred and twenty larvae were fed copper hydroarsenate-arsenite, the doses ranging from 0.0066 to 0.378 mg./gm. body weight. Only one individual in this group, which consumed 0.144 mg. gm. body weight, died in 72 hours.

There was no significant mortality among the controls on untreated and starch-coated leaves. A summary of the remaining data is given in table 3.

Differential Grasshopper.—The single-leaf method was not applicable to this insect. Instead, a method of feeding poisoned bait (Richardson & Seiferle 1938) was employed. The bait consisted of moist whole wheat bran mixed with from 0.02 to 10.0 per cent of the poison. Approximately one gram of the bait was presented to a previously weighed insect in a stoppered cylinder, and the insect was permitted to feed *ad libitum*. The loss in weight of the bait was determined after feeding ceased. Water-loss checks showed that, without feeding, the bait lost about one per cent of its weight during the feeding period. The individual dose was calculated on a basis

Table 2.—Toxicity of four arsenicals to fifth-instar imported cabbage worms.

Material	No. Insects	LD50±5% Limits (Mg./gm. Body Weight)	Slope± Standard Error	Chi-Square	P
Copper hydroarsenate	120	0.45 ±.07	4.37± .93	4.41	.11
Copper hydroarsenate-arsenite	85	0.24 ±.02	10.19±2.33	1.46	.24
Lead arsenate	197	0.21 ±.07	1.79± .42	2.44	.49
Paris green	100	0.013±.003	3.54± .67	1.31	.53

Table 3.—Toxicity of three arsenicals to fifth-instar corn earworms.

MATERIAL	No. INSECTS	LD50±5% LIMITS (MG./GM. BODY WEIGHT)	SLOPE± STANDARD ERROR	CHI-SQUARE	P
Lead arsenate	110	0.17 ±.038	3.61±0.81	4.18	.13
Calcium arsenate	48	0.21 ±.083	2.46±0.99	0.45	.83
Paris green	28	0.023±.006	5.50±1.85	1.55	.22

of milligrams per gram of body weight. Both sexes were used; the average weight of females was 1.45 grams while males averaged 0.88 gram. In spite of correction for difference in body weight, there seemed to be a sex difference in susceptibility. Accordingly, the data in table 4 are broken down to show results for each sex. Mortality data were taken over a period of five days; after this period, a live grasshopper was considered unaffected. The field-collected insects were relatively free from parasites and disease.

Sixty-five grasshoppers were fed lead arsenate but the effect produced by this poison was very slight. Thirteen adults ingested over 10 mg. of poison per gram of body weight with only seven of them dying.

Spotted Cucumber Beetle.—A quantitative individual dosage method was not used in determining the toxicity of the arsenicals to this insect, but rather, one (Richardson & Glover 1932) that indicated relative speed of toxic action. Since this method depends on the oral ingestion of poison when the insects clean their appendages and on penetration through the cuticle, actual dose per insect was not determined. Ten adults were rolled in one gram of dust for ten seconds and then caged over foliage. The criterion of death was the failure of leg movement when the beetles were probed. Counts on living and dead beetles were made at hourly intervals for the first two hours, thereafter at two hour intervals until eight hours had passed. Additional records were taken when necessary at 12, 24, 48, and 72 hours. Results for each dust were determined on 200 beetles. The beetles after treatment were held at laboratory temperatures which varied between 25° and 28° C. In addition to tests with the unmixed materials, comparable tests were made with each material diluted with starch to give concentrations of 25, 50, and 75 per cent. Starch alone had no effect on the beetles. The experimental data were plotted on graph paper and the time required to kill fifty per cent of the beetles was read from the graphs. The results are given in table 5.

PHYTOTOXICITY TESTS.—A series of laboratory experiments was made to determine the relative plant tolerance of the seven arsenicals. A humid chamber technique (McGovran & Farrar 1936) was employed. The London Horticultural variety of cranberry bean was the only plant used. Two pairs of seed leaves or four leaves were used in each test, and three such lots of plant material, each lot in a bottle containing water, were tested with each concentration of an arsenical. The leaves were dipped in a suspension of the arsenical until they were coated uniformly and held for twenty-four hours in the humid cabinet. After removal from the cabinet, the leaves were allowed to dry before the injured area was estimated. The results with five concentrations of each material are given in table 6.

Table 4.—Toxicity of four arsenicals to adult differential grasshoppers.

MATERIAL	No. INSECTS		LD50±5% LIMITS (MG./GM. BODY WEIGHT)		SLOPE±STANDARD ERROR		CHI-SQUARE AND P-			
	Female	Male	Female	Male	Female	Male	Female		Male	
Copper hydroarsenate	30	24	0.16 ±.055	0.11 ±.051	3.78±1.23	2.76±1.08	0.70	.75	1.62	.76
Copper hydroarsenate-arsenite	29	29	0.10 ±.053	0.068±.024	2.43± .85	3.75±1.19	2.02	.65	1.16	.74
Calcium arsenate	34	29	0.18 ±.12	0.16 ±.086	2.21± .82	2.82± .93	0.66	.86	2.70	.26
Paris green	32	29	0.048±.016	0.022±.010	3.51±1.12	3.28±1.15	1.68	.75	1.79	.62

Table 5.—Relative speed of toxicity of seven arsenicals to spotted cucumber beetle.

Material	Concentration of Dust			
	25%	50%	75%	100%
	Minutes Required to Kill 50% of Beetles Treated			
Copper hydroarsenate	250	145	135	100
Copper hydroarsenate-arsenite	195	125	90	80
Lead arsenate	600	365	310	245
Calcium arsenate	210	145	90	80
Paris green	225	95	65	55
Paris green-calcium arsenate	180	140	65	60
Cupric metaarsenite	205	150	125	85

Table 6.—Phytotoxicity tests with seven arsenicals.

Material	Concentration of Materials				
	0.05%	0.1%	0.125%	0.2%	0.3%
	Percentage of Leaf Area Injured				
Copper hydroarsenate	Trace	2	4	4	1
Copper hydroarsenate-arsenite	Trace	1	5	8	2
Lead arsenate	Trace	3	4	20	30
Calcium arsenate	19	56	65	53	72
Paris green	70	100	100	100	100
Paris green-calcium arsenate	20	62	38	81	93
Cupric metaarsenite	7	90	61	96	99

Copper hydroarsenate and copper hydroarsenate-arsenite appear to have very little effect on the foliage of this bean variety.

DISCUSSION OF RESULTS.—*Colorado Potato Beetle.* Owing to repellence, the tests with cooper hydroarsenate-arsenite provided insufficient data for determining an interpolated LD50. Copper hydroarsenate showed less repellent action but its toxicity was lower than any of the remaining five materials tested. Paris green and cupric metaarsenite proved most toxic. The reacted paris green-calcium arsenate product and calcium arsenate were approximately equal in toxicity, and they were significantly more toxic than lead arsenate.

Imported Cabbage Worm.—Copper hydroarsenate-arsenite was markedly more toxic to cabbage worms than copper hydroarsenate. Both of these products, however, were much less toxic than Paris green. Lead arsenate and copper hydroarsenate-arsenite have somewhat similar toxic properties. As stated previously, calcium arsenate was quite ineffective.

Corn Earworm.—Copper hydroarsenate and the arsenate-arsenite product were so repellent to the larvae that zones of toxicity could not be determined. Paris green was very toxic while calcium arsenate and lead arsenate showed about equal toxic action. Lead arsenate was noticeably repellent to the larvae.

Differential Grasshopper. Tests with copper hydroarsenate-arsenite against this species of grasshopper provided data for determining an LD50 which was lower than the comparable figure for copper hydroarsenate, but the five per cent limits of the two materials overlap. Both of the new copper arsenical products were more toxic than lead arsenate. The LD50s for the new products were lower than those for calcium arsenate but the differences were not significant. Paris green was more toxic to male grasshoppers than any of the materials tested, but against female insects it was not significantly different from copper hydroarsenate-arsenite.

The statistical analysis supports, to a certain degree, the observed sexual difference in response to the arsenicals. All the LD50s for the males were lower than those for the females; but except for Paris green, the differences were not statistically significant.

Spotted Cucumber Beetle.—Lead arsenate proved least effective of the materials tested against this beetle. Copper hydroarsenate was decidedly more toxic than lead arsenate, yet inferior to the other five materials tested. The remaining compounds gave rather similar results with Paris green showing the best overall performance.

Phytocidal tests reveal that the two new copper arsenical mixtures injure bean leaves less than lead arsenate which is usually considered the safest of the commercial arsenicals for use on foliage.

SUMMARY.—Copper hydroarsenate and copper hydroarsenate-arsenite, two new basic arsenical products, were compared toxicologically on a weight basis with certain standard arsenical compounds. Relative toxicities were determined on Colorado potato beetle larvae, imported cabbage worms, corn earworms, adult differential grasshoppers, and adult spotted cucumber beetles. A quantitative individual dosage technique was employed on the first four insects mentioned.

Greatest susceptibility to copper hydroarsenate and copper hydroarsenate-arsenite was shown by the grasshoppers followed by potato beetle larvae, cabbage

worms, and corn earworms. Copper hydroarsenate was more toxic than the arsenate-arsenite to potato beetles but proved less effective against cabbage worms and spotted cucumber beetles. There was no significant difference in the action of these materials on grasshoppers. While both materials had little effect on corn earworms, the data were not sufficient to indicate relative toxicity.

A comparison of the new copper arsenical products with calcium arsenate shows the former to be more effective against the cabbage worm. All three materials had similar toxic effects on the grasshoppers, while calcium arsenate was definitely more toxic to potato beetle larvae and corn earworms. Against the spotted cucumber beetle, calcium arsenate was about as toxic as the arsenate-arsenite product but more toxic than copper hydroarsenate.

Lead arsenate was less effective than the new copper arsenical products on grasshoppers and spotted cucumber beetles. It was more effective, however, when fed to potato beetle larvae and corn earworms. Against cabbage worms, the copper hydroarsenate-arsenite product and lead arsenate showed similar toxicity, and both were more toxic than the copper hydroarsenate product.

Paris green proved to be the most toxic compound against all insects tested. Two special arsenicals, the reacted paris green-calcium arsenate product and cupric metaarsenite were tested against two insects and were found to be nearly as toxic as paris green.

Experiments with potato beetle larvae and corn earworms revealed a definite repellent effect of the new copper arsenicals with hydroarsenate-arsenite apparently having the greater repelling property. Phytotoxicity tests in the laboratory showed that the new copper arsenicals were much less injurious to bean foliage than the other arsenicals.—6-5-44.

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The pink bollworm quarantine and regulations were revised effective May 1, 1944, to extend the regulated areas to include recently found infestations in 3 parishes in southwestern Louisiana, 4 counties in the Coastal Bend of Texas, and 1 county in western Texas; also to release from quarantine an area in the Panhandle of Texas and an adjacent county in New Mexico in which no specimens of the bollworm have been taken in recent years.

Tennessee quarantine revisions which became effective June 15, 1944, included: (1) Extension of the European corn borer regulated area, and (2) redesignation of sweetpotato weevil infested areas and lifting the former provision for the acceptance from such area of sweetpotato tubers when fumigated. The current quarantine prohibits the entry of the tubers and plants from the regulated areas and requires inspection certification as to shipments from nonregulated areas of the infested States.

Further Tests of Synthetic Organic Compounds as Insecticides

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In a previous paper (Swingle, *et al.* 1944) results were given of certain tests to determine the insecticidal effectiveness of a number of synthetic organic compounds, conducted at the Sanford, Fla., laboratory. The present paper represents a continuation of this work, giving data on 64 additional compounds. Other papers will appear as results on other compounds become available.

Most of the materials tested were synthetic organic compounds supplied by the Division of Insecticide Investigations and prepared under the general direction of R. C. Roark and H. L. Haller. Most of the samples were crystalline solids supplied with little or no conditioning to obtain a particle size as small as is customary with commercial insecticides, and it was not considered practical to grind all these samples.

Seventeen species of insects were used in these tests. Most of the insects were reared in the laboratory to secure uniformity in age and physical condition, as well as availability when desired.

The methods of testing have been described by Swingle *et al.* (1941). Preliminary tests were made by feeding insects excised foliage which had been rather heavily dusted with the compound. The tests were conducted in closed Petri dishes, which permitted action as a contact or stomach insecticide or as a fumigant.

Of the 64 compounds reported in this paper, 14 were toxic to at least a few of the insects on which they were tested. The results of the preliminary tests on the toxic materials are summarized in table 1. Since additional tests have been made on these various compounds, each compound will be discussed separately. The remaining 50 compounds, being ineffective against from 5 to 8 different species, are considered unpromising as a group, and are listed by name only.

p-Anisidine.—Table 1 shows that this compound was rather effective against the southern armyworm, repellent to the bean leaf roller and Hawaiian beet webworm, but not effective against the remaining insects. In a volatility test made by weighing a dust deposit on a glass slide, the deposit was found to have entirely volatilized within 5 days. In fumigation tests, however, the compound was only slightly toxic to larvae of the southern army worm and the Hawaiian beet webworm. As a contact dust it killed 82 per cent of nearly grown nymphs of the American cockroach in a 3-day exposure. Mixed with grain at 1 to 1000 parts by weight, it killed all the cowpea weevils confined in the grain, but was apparently nontoxic to adults of the rice weevil. When mixed in sand at the same rate, the compound killed 100 per cent of adult termites, *Reticulitermes* sp., in 4 days, but at 1 to 5000 it killed only 34 per cent of the termites.

4-Bromoacetophenone.—This compound was very toxic in the tests reported in table 1. However, a dust deposit was found to be completely volatilized in 5 days. In fumigation tests with fourth instars of five lepidopterous species, the compound killed all the insects tested, indicating that the results in table 1 may have been due to fumigation. A saturated atmosphere in half-pint jars killed only 62 per cent of adults of the cowpea weevil and 38 per cent of the rice weevil. Mixed with sand at 1–1000, the compound killed 100 per cent of adult termites, and at 1–5000, 80 per cent. As a contact dust it killed 100 per cent of American cockroaches and first-instar squash bugs. Mixed with grain at 1–1000 parts by weight, the compound killed all adults of the rice weevil and cowpea weevil. As a contact spray (1 per cent suspension) it was not effective against second instar squash bugs, large nymphs of the pea aphid, or fourth instars of the Hawaiian beet webworm or southern armyworm.

p-Bromo-N-ethylbenzenesulfonamide.—This compound was very effective against four of the five insects listed in table 1. Since a dust deposit was found to be not volatile, a 1 per cent spray suspension was applied to plants, which were then fed to insects. All larvae of the Hawaiian beet webworm, melon worm, southern army-

Table 1.—Results of preliminary tests of compounds found to be toxic to certain species of insects when applied as a dust to foliage.

COMPOUND	INSECT[1]	DEPOSIT	MORTALITY AFTER 2 DAYS	FEEDING ON FOLIAGE
		Micrograms per sq. cm.	*Per cent*	
p-Anisidine $CH_3OC_6H_4NH_2$	Bean leaf roller	435	33	Slight
	Blister beetle	250	10	Moderate
	Cross-striped cabbage worm	355	0	Much
	Hawaiian beet webworm	310	32	Slight
	Melon worm	435	40	Moderate
	Southern armyworm	355	100	None
	Southern beet webworm	310	26	Moderate
4-Bromoacetophenone $BrC_6H_4COCH_3$	Hawaiian beet webworm	155	76	Slight
	Melon worm	155	100	None
	Southern armyworm	365	100	None
	Southern beet webworm	265	100	None
p-Bromo-N-ethylbenzenesulfonamide $BrC_6H_4SO_2NHC_2H_5$	Blister beetle	215	96	Slight
	Cross-striped cabbage worm	225	4	Moderate
	Melon worm	250	92	None
	Southern armyworm	510	100	Slight
	Southern beet webworm	510	100	Slight
p-Bromo-N-isobutylbenzenesulfonamide $BrC_6H_4SO_2NHC_4H_9$	Blister beetle	185	24	Slight
	Hawaiian beet webworm	220	20	Moderate
	Melon worm	230	16	Slight
	Southern armyworm	250	4	Moderate
	Southern beet webworm	250	81	Slight
p-Bromo-N-propylbenzenesulfonamide $BrC_6H_4SO_2NHC_3H_7$	Blister beetle	260	92	Slight
	Cross-striped cabbage worm	270	0	Moderate
	Melon worm	125	72	Slight
	Southern armyworm	300	60	Slight
	Southern beet webworm	300	75	Slight
4-Bromo-o-toluidine $BrC_6H_3(CH_3)NH_2$	Bean leaf roller	355	43	None
	Blister beetle	200	18	Moderate
	Cross-striped cabbage worm	560	100	Slight
	Hawaiian beet webworm	540	92	Slight
	Melon worm	355	100	None
	Southern armyworm	560	100	None
	Southern beet webworm	540	57	Slight
4,6-Dinitro-o-cresol propionate $CH_3C_6H_2(NO_2)_2COOC_2H_5$	Bean leaf roller	315	80	Slight
	Blister beetle	185	100	Slight
	Melon worm	315	90	Slight
	Okra caterpillar[2]	405	100	None
	Polkadot wasp moth[3]	405	67	None
	Southern armyworm	315	100	Slight
	Southern beet webworm	230	78	Slight
2,4-Dinitrophenol propionate $(NO_2)_2C_6H_2COOC_2H_5$	Blister beetle	310	100	Slight
	Cross-striped cabbage worm	195	100	None
	Hawaiian beet webworm	230	100	None
	Southern beet webworm	550	79	Moderate
1,4-Diphenylthiosemicarbazide $C_6H_5NHNHCSNHC_6H_5$	Bean leaf roller	255	0	Moderate
	Cross-striped cabbage worm	108	0	Much
	Hawaiian beet webworm	75	29	Slight
	Melon worm	255	48	Slight
	Southern armyworm	75	13	Moderate
	Southern beet webworm	170	60	Slight
2-Furanacrolein $OCHCHCHCCHCHCHO$	Hawaiian beet webworm	295	34	None
	Imported cabbage worm	295	40	Slight
	Melon worm	230	28	Slight
	Southern armyworm	310	0	Much
	Southern beet webworm	370	86	None

Table 1.—*Continued*

Compound	Insect[1]	Deposit	Mortality After 2 Days	Feeding on Foliage
		Micrograms per sq. cm.	*Per cent*	
2-Methylcyclohexanone semicarbazone $C_6H_9(CH_3)=NNHCONH_2$	Blister beetle	200	95	Moderate
	Cross-striped cabbage worm	140	76	Moderate
	Melon worm	385	20	Much
	Southern armyworm	385	83	Moderate
	Southern beet webworm	235	94	Moderate
4-Methylcyclohexanone semicarbazone $C_6H_9(CH_3)=NNHCONH_2$	Blister beetle	230	100	Moderate
	Cross-striped cabbage worm	385	100	Slight
	Melon worm	350	60	Slight
	Okra caterpillar[2]	210	68	Moderate
	Southern armyworm	350	100	Slight
	Southern beet webworm	255	100	Moderate
beta-Naphthonitrile $C_{10}H_6CN$	Hawaiian beet webworm	185	36	None
	Imported cabbage worm	185	72	Slight
	Melon worm	185	4	Slight
	Southern armyworm	250	37	Moderate
	Southern beet webworm	230	37	Slight
	Yellow woolly bear	265	100	None
1-Phenylthiosemicarbazide $C_6H_5NHNHCSNH_2$	Bean leaf roller	495	30	Slight
	Blister beetle	225	0	Slight
	Cross-striped cabbage worm	185	0	Much
	Hawaiian beet webworm	215	92	Slight
	Melon worm	495	60	Slight
	Southern army worm	215	20	Moderate
	Southern beet webworm	130	52	Moderate

[1] Blister beetle, *Epicauta lemniscata* F., used as adult; all others as fourth or fifth instar.
[2] *Anomis erosa* Hbn.
[3] *Syntomeida epilais* Walk.

worm, and southern beet webworm died, but only 40 per cent of the cross-striped cabbage worms, which fed moderately on the sprayed foliage. Foliage treated with a 0.5 per cent spray killed all larvae of the melon worm and southern armyworm, but only 37 per cent of adult blister beetles, although only slight feeding occurred. A 1 per cent spray suspension, applied to young plants in two treatments, caused severe injury to bean, potato, pumpkin, and swiss chard. Only very slight injury occurred on lettuce and broccoli, but in general the material appears to be unsafe for use on foliage. As a contact dust the compound killed 90 per cent of American cockroaches in 3 days of exposure.

p-Bromo-N-isobutylbenzenesulfonamide.—The isobutyl-substituted compound was not so toxic as the ethyl form just discussed. Although rather repellent, it was only moderately toxic to the insects in table 1. This compound was also nonvolatile and was tested against a variety of insects as a spray on foliage. A 1 per cent spray suspension killed 33 per cent of larvae of the cross-striped cabbage worm, 90 per cent of the southern armyworms, and 100 per cent of the Hawaiian beet webworms, melon worms, and southern beet webworms. A 0.5 per cent spray was not effective against blister beetle adults, but killed 50 per cent of southern armyworm and 100 per cent of melon worm larvae. Two applications of a 1 per cent spray on young plants caused no injury to lettuce, broccoli, potato, and pumpkin, but did cause slight injury to bean and swiss chard.

p-Bromo-N-propylbenzenesulfonamide.—This compound was also less toxic than the ethyl compound reported in table 1, but it was considerably more effective than the isobutyl member of the series. Being nonvolatile, it was tested as a spray against larvae of leaf-feeding insects, and a 1 per cent suspension was found to kill 87 per cent of the cross-striped cabbage worms, 93 per cent of the melon worms, and 100 per cent of the cabbage loopers, imported cabbage worms, and southern armyworms. A 0.5 per cent suspension killed only 12 per cent of blister beetle adults, but 100 per cent of the melon

worm larvae and 87 per cent of the southern armyworm larvae. Two applications of a 1 per cent spray suspension on young plants caused no injury to lettuce, slight injury to broccoli and potato, and moderate to severe injury to bean, pumpkin, and swiss chard. Applied as a dust, the compound also killed 100 per cent of nearly full grown cockroaches.

4-Bromo-o-toluidine.—This compound was toxic to most of the insects reported in table 1, but it was also completely volatilized in 2 days. An atmosphere saturated with the vapor killed from 0 to 80 per cent of five species of lepidopterous larvae, showing that the compound has toxicity as a fumigant, but some contact or stomach action must also take place to account for the generally greater mortality reported in table 1. It was practically nontoxic as a fumigant against the rice weevil and cowpea weevil. When the compound was mixed with wheat at 1–1000 parts by weight, it killed all the adult rice weevils confined in it. Mixed in sand at the same concentration, it killed 100 per cent of adult termites, and at 1–5000 parts by weight it killed 95 per cent of the termites. As a contact dust it killed 100 per cent of nearly grown cockroaches.

4,6-Dinitro-o-cresol propionate.—In previous work 4,6-dinitro-o-cresol has been found very toxic to insects. This salt of 4,6-dinitro-o-cresol is also very toxic, as shown in table 1. As the compound is not volatile, spray suspensions were applied to foliage for testing as a stomach insecticide. A 1 per cent suspension killed 100 per cent of adult blister beetles, 96 per cent of larvae of the Hawaiian beet webworm and melon worm, and 56 per cent of the southern armyworm larvae. A 0.5 per cent suspension killed 96 per cent of adult blister beetles. A 0.5 per cent spray suspension applied to young plants caused severe injury to bean, pumpkin, okra, and collards, but not to corn. In treated grain at a concentration of 1–1000 by weight, the compound killed 100 per cent of the cowpea weevils and 79 per cent of the rice weevils. A contact dust killed only 80 per cent of nearly grown cockroaches, which is a considerable reduction in toxicity from that of dinitro-o-cresol itself. Mixed with sand at 1–5000, the compound killed 100 per cent of termites.

2,4-Dinitrophenol propionate. — This compound, which is similar in structure to the preceding compound, was also very toxic in the tests reported in table 1. It was found very toxic as a spray, but was so injurious to foliage that it could not be used on plants. As a contact dust it killed 100 per cent of nearly grown cockroaches in 24 hours. In sand it killed all termites at 1–1000 parts by weight, and 96 per cent at 1–5000.

1,4-Diphenylthiosemicarbazide. — This compound is similar in structure to 1,4-diphenylsemicarbazide (Gahan *et al.* 1941) with sulfur replacing the oxygen. This substitution has reduced the toxicity. In addition to the results in table 1, tests against cockroaches, cowpea weevils, rice weevils, and termites showed little or no toxicity.

2-Furanacrolein.—All the insects in table 1 except the armyworm showed some susceptibility to this compound. In most cases, however, the action was characterized by very limited feeding rather than any appreciable mortality. Since the compound was found to be approximately 80 per cent volatile in 2 days, these insects were also subjected to fumigation tests. In these tests the mortality was 100 per cent with the Hawaiian beet webworm and melon worm, 76 per cent with the cross-striped cabbage worm, 92 per cent with the southern armyworm, and 88 per cent with the southern beet webworm. In fumigation tests in half-pint jars against the cowpea weevil and rice weevil, the mortalities were 15 and 79 per cent, respectively. A test against the cockroach resulted in 40 per cent mortality.

2-Methylcyclohexanone semicarbazone.—This compound was fairly toxic as a dust applied to foliage (table 1), but permitted more feeding than did some of the other toxic compounds. As it was not volatile, it was tested as a spray on foliage. A 1 per cent suspension was not toxic to the blister beetles, killed 100 per cent of the cross-striped cabbage worms and Hawaiian beet webworms, 73 per cent of the melon worms, and 97 per cent of the southern armyworms. A 0.5 per cent suspension was not toxic to the melon worms but killed 83 per cent of the southern armyworms. Two applications of a 1 per cent spray on young plants caused no injury to bean, broccoli, and swiss chard, but did cause slight injury to lettuce, pumpkin,

and potato. The compound was not effective against cockroaches or termites with the methods used.

4-Methylcyclohexanone semicarbazone.—This isomeric form was more toxic than the preceding compound. A 1 per cent spray suspension on foliage killed 100 per cent of the blister beetles, cross-striped cabbage worms, and Hawaiian beet webworms, 52 per cent of the melon worms, and 79 per cent of the southern armyworms. A 0.5 per cent spray killed 96 per cent of the blister beetles, 13 per cent of the melon worms, and 91 per cent of the southern armyworms. Two applications of a 1 per cent spray on growing plants caused no injury to six varieties of truck crops. This is an interesting case of an isomeric form showing higher toxicity to insects but lower toxicity to plants.

Beta-Naphthonitrile.—This compound had only slight to moderate toxicity. It is too volatile (30 per cent in 2 days) for use as a stomach insecticide on foliage, but not sufficiently so to act as a fumigant. When mixed with grain at 1–1000, it killed 100 per cent of the cowpea weevils used and 26 per cent of the rice weevils. It also killed 100 per cent of termites when mixed with sand at 1–5000. It was not effective against the American cockroach.

1-Phenylthiosemicarbazide.—The toxicity of this compound was about equal to that of the diphenylthiosemicarbazide. Neither appeared to be sufficiently toxic for use as a practical insecticide A 1 per cent spray suspension caused 94 per cent mortality of the Hawaiian beet webworm, the most susceptible insect in the dusting tests (table 1). The compound was not toxic as a dust to cockroaches, or, mixed with seed, against the cowpea weevil and the rice weevil. When mixed with soil at 1–1000 it killed 100 per cent of adult termites, but this mortality may have been a result of dessication, as the repellent properties of the material kept the insects from entering the soil.

COMPOUNDS FOUND TO HAVE LITTLE OR NO TOXICITY.—Fifty additional compounds were tested against from five to eight species of insects, but were found to have little or no toxicity by the methods used.

Acetone-levo-menthydrazone
Adipic acid
1-Amino-2-naphthol-4-sulfonic acid
5-Aminosalicylic acid hydrochloride
Benzimidazole
Benzoic acid
Benzoic anhydride
N′(alpha-Benzoyl) benzylidine-N,N-dimethyl-p-phenylenediamine
o-Benzoyllactic acid
Bis(4-acetylaminophenyl)-sulfone
Bis(4-aminophenyl) sulfide
Bis(4-aminophenyl) sulfone
1,3-Bis(p-tolyl) triazine
2-Bromoanthraquinone
o-Bromobenzoic acid
p-Bromo-N-butylbenzenesulfanomide
2-Bromo-3-nitrobenzoic acid
p-*tert*-Butylphenol
o-Chlorobenzoic acid
p-Chlorobenzoic acid
p-Chloro-alpha-isonitrosoacetanilide
5-(p-Chlorophenyl)-5-methylhydantoin
Cinnamic acid
Coumarilic acid
4,4′-Diaminobenzophenone
2,3-Dihydroxyquinoxaline
5,5-Diisopropylhydantoin
1-(2,4-Dinitrophenyl)-4-methylthiosemicarbazide
1,5-Diphenyl-2,4-pentadien-1-one
1,5-Diphenyl-3-thiocarbohydrazide
sym-Di-o-tolythiourea
2-Furanacrylic acid
p-Hydroxypropiophenone
4′-Methyl-3,4-methylenedioxychalcone
3,4-Methylenedioxycinnamic acid
5-Methyl-5-phenylhydantoin
4-alpha-Naphthyl-1-phenylsemicarbazide
4-alpha-Naphthyl-1-phenylthiosemicarbazide
4-alpha-Naphthyl-1-p-tolylthiosemicarbazide
3-Nitrophthalic anhydride
Oxamide
5-Pentamethylenehydantoin
N-Phenylphthalimide
1-Phenyl-4-o-tolylsemicarbazide
1-Phenyl-4-o-tolylthiosemicarbazide
Phthalamide
Piperonyloin
2,4-Toluenediamine
alpha,alpha, beta-trichlorobutyraldehyde hydrate
o-Veratraldehyde semicarbazone

SUMMARY.—Sixty-four synthetic organic compounds were tested for insecticidal action against from four to eight species of economic insect pests. Fourteen were toxic to at least a few species and are discussed in some detail. The most toxic compounds were 4-Bromoacetophenone, a volatile fumigant and contact insecticide; p-bromo-N-ethylbenzenesulfonamide, a fairly toxic stomach insecticide but injurious to foliage; 4,6-dinitro-o-cresol propionate and 2,4-dinitrophenol propionate, very toxic compounds but injurious to foliage; and 4-methylcyclohexanone semicarbazide, a fairly toxic stomach insecticide but injurious to foliage. Fifty compounds were relatively nontoxic and are listed by name only.

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The Repellency of a Pyrethrum-Thiocyanate Oil Spray to Flies Attacking Cattle

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In 1942, a cooperative experiment was set up between the Departments of Entomology and Dairy of the Oklahoma Agricultural Experiment Station and the *Continental Oil Company*, the purpose of which was to determine the effect of spraying milk cows for fly control on milk production. The experiments were carried out from May to early September, 1942, inclusive. The spray formula used was a blend of pyrethrum concentrate (2 per cent), *Lethane 384* (3 per cent), *Lethane 384 Special* (1 per cent), in oil.[2] Considerable information was obtained on the repellent effect of this spray on the stablefly, *Stomoxys calcitrans* (L.), and the hornfly, *Haematobia irritans* (L.), during the time the animals were in the pasture. In addition, information was also obtained on the comparative abundance of these species at different intervals throughout the day. This paper deals entirely with the entomological phase of the experiment.

METHODS.—The experiment was divided into two parts. The object of the first was to determine the repellency of one rate of spraying on these species of flies. The object of the second was to determine comparative repellency of this spray formula when two rates of application were given. In the first experiment, 20 cows were selected. This group consisted of 6 Holstein-Freisians, 6 Ayrshires, 4 Guernseys, and 4 Jerseys. The cows were divided into two groups as nearly uniform in relation to fly susceptibility as possible, based on counting the average number of flies of both species observed on each animal in the pasture at various intervals during the day. Group 1 was sprayed from May 14, to June 7, and again from July 5 to August 29. During this period, Group 2 was not sprayed. From June 8, to July 4, the animals in Group 2 were sprayed, during which period those in Group 1 were used as a check. Alternate spraying in these groups was to equalize variability in milk production. Each animal in the sprayed group was sprayed at five A. M. and five P. M. just preceding milking. The check animals were milked and removed from the barn before any spraying was done and the amount of spray used per cow was determined by trial at the start of the test. It was found that about 15 cc. of spray material was sufficient to cover adequately all parts of the cow, including feet and legs, with a thin film of the spray. During the first period all cows received the same amount of spray. This was applied evenly over the entire animal with the possible exception of the head, which received a small amount. The spraying was done from front to back. A *Vestal* electric sprayer was used which delivered 15 cc. of spray in approximately 35 seconds. Beginning with the late June period, the cows were sprayed according to body surface area rather than using an equal amount of spray material for each cow regardless of size. Elting's formula was

[1] Contribution from the Dairy and Entomology Departments, Oklahoma A. & M. College, and the *Continental Oil Company*.

[2] Base oil analysis:—I.B.P. 484F; end point 714F gravity A.P.I. 34.7; viscosity s.a.u. at 100 calulated from modified Ostwald 45.9; unsaturates 6 per cent.

Pyrocide 20 deodorized-clarified, product of McLaughlin-Gormley-King Co., contains 2.0 grams pyrethrins per 100 cc. (Seil method) equivalent to 2.5 per cent pyrethrins by weight, made up with a special deodorized mineral base.

Lethane 384 and *Lethane 384 Special*, made by Rohm and Haas Co., *Lethane 384* is a solution of beta butoxy beta thiocyano diethyl ether standardized at 50 per cent by volume with a highly refined hydrocarbon oil, *Lethane 384 Special* is a mixture of organic thiocyanates including beta butoxy beta thiocyano diethyl ether and the thiocyano ethyl esters of higher fatty acids standardized at 50 per cent thiocyanate content by volume with a highly refined hydrocarbon oil.

used to determine surface area. This formula is $SA = 1470 \times W^{0.56}$ in which SA is the surface area in square centimeters and W the live weight in kilograms. The spray was applied at approximately the rate of 1 cc. for each 3.2 square feet of surface area.[1] Thus, the body surface area of a small Jersey equalling 35.53 square feet would receive 11 cc. of spray and a large Holstein with a surface area of 61.37 square feet, 19 cc. Other cows received an amount between these two extremes. This resulted in a very little change in the amount of spray used on an average sized animal and in the total amount used on the group.

In the second experiment, 24 cows were used, divided into four groups of six each. The same breeds were used in this test. One group was sprayed lightly at the rate of 0.5 cc. for each 3.23 square feet of surface area, which was about one-half the rate previously used. An average sized cow thus received about 7.5 cc. of spray twice daily. The smallest Jerseys received about 5 cc. of spray and the largest Holstein 10 cc. Another test group was sprayed evenly at the rate of about 2 cc. per 3.23 square feet of surface area per spray application. Two groups of 6 cows each were used as checks. This test was divided into 2 periods of 2 weeks each. During the second period, the check animals were sprayed while those treated during the first period were held as checks.

Following morning spraying the animals were returned to the pasture and at regular intervals from 7 A.M. to 3 P.M. separate counts of horn- and stableflies were taken on each animal. The animals were then driven to the barn and at 3:30 P.M. a count was taken in the lot near the barn. A 4:30 P.M. count was taken in the barn before spraying. Following the afternoon milking and spraying, the animals were returned to the pasture and additional counts made, at 6:15 and 7:15 P.M. during the first experiment; at 6:15, 7:15 and 8:15 P. M. in the second experiment.

REPELLENCY OF 1 CC. SURFACE UNIT.—The results of the first test are shown in table 1. There were 16 days of complete records from June 26, to August 1, inclusive. These records are mostly from one spraying period during which the sprayed cows were those which had been found to be less attractive to both species of flies in earlier tests. This fact gave the sprayed animals an advantage. Correcting the data to take this into consideration did not make any material difference in the results. The spray was repellent to hornflies at all times. At 10:00 A. M., or five hours after spraying, repellency had disappeared for the stablefly and there was but slight repellency to this species at four hours after spraying.

[1] The unit used was 1 cc. for each 3000 square centimeters of surface area.

Table 1.—Repellency of a pyrethrum-thiocyanate-oil spray to horn- and stableflies.

TIME	PER CENT HORNFLIES OBSERVED ON SPRAYED COWS	PER CENT STABLEFLIES OBSERVED ON SPRAYED COWS
7	7.5	23.6
8	10.7	31
9	15.6	43.2
10	21.9	45.5
11	27.4	45.6
12	28.9	51.3
1	40.2	47.6
2	42	49.3
3	43.6	50.4
3:30	43.4	50.2
6:15	12.1	21.4
7:15	22.8	35.1

COMPARATIVE REPELLENCY OF 0.5 CC. AND 2 CC.—The results of the second test are presented in table 2. The four groups of six cows each were composed of animals selected so that the milk production was comparable. However, there was considerable difference between groups as

Table 2.—Repellency of a pyrethrum-thiocyanate-oil spray to hornflies and stableflies at 0.5 cc. and 2 cc. per 3.23 sq. ft.

TIME	PER CENT FLIES OBSERVED ON COWS SPRAYED AT 0.5/3.23		PER CENT FLIES OBSERVED ON COWS SPRAYED AT 2/3.23	
	Horn-flies	Stable-flies	Horn-flies	Stable-flies
7	11.8	27.8	6.2	18.8
8	19.2	28.1	8.1	16.7
9	24.4	33.3	10.7	35.3
10	28.9	35.3	17.9	34.1
11	29.1	46.9	20.9	39.2
12	32.6	45.1	29	37.0
1	34.6	40.6	33.3	34.0
2	34.5	36.1	39.2	31.9
3	32.3	39.3	41.5	34.1
3:30	33.6	39.0	37.5	30.9
6:15	6.7	12.3	6.9	2.0
7:15	22.5	30.6	22.2	25.9
8:15	24.4	25.5	20.3	23.7

to fly attractiveness. Twelve complete records were obtained for this test from August 8–29 inclusive. Again a majority of the records are within one group period of spraying so that in 9 records out of the 12, cows receiving the lighter application were less attractive to both species and were more attractive for 3 days. There was, therefore, a definite advantage to the lighter spray application.

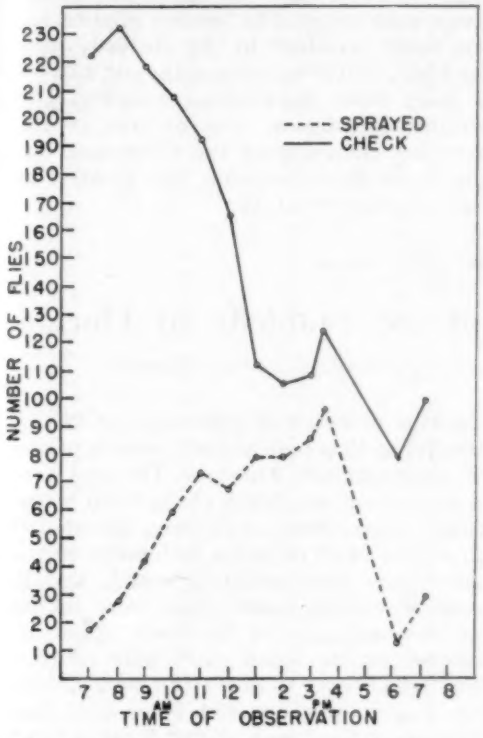

FIG. 1.—Comparative numbers of hornflies on sprayed and unsprayed cows, June 26–Aug. 1, 1942.

The lighter application was definitely repellent to the hornfly at all times, but the degree of repellency became less as the time interval from spraying increased. Against the stablefly the lighter application was also repellent up to 10:00 A. M., or 5 hours after spraying.

The heavier application was more repellent to the hornfly up to noon or 7 hours after spraying. At other times, no significant differences were observed. The heavier application was more repellent to the stablefly for 4 hours after the morning spraying and for 2 hours after the evening spraying. The differences are probably greater than shown because, as previously stated, the lighter sprayed cows were less attractive as a group to both species of flies.

REPELLENT EFFECT OF SPRAYS.—During the first tests there was a steady and marked decline in the hornfly infestation on the unsprayed animals from 8:00 A. M. up to 2:00 P. M. (Fig. 1). A temporary rise began at 3:00 P. M. and reached its peak at 3:30 P. M. On the

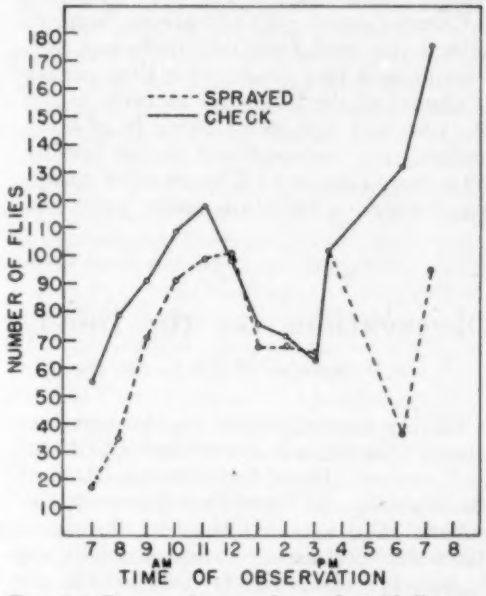

FIG. 2.—Comparative numbers of stableflies on sprayed and unsprayed cows, June 26–Aug. 1, 1942.

sprayed animals the infestation increased as the repellency declined following the morning spraying. This increase continued up to 3:30 P. M. A sharp drop was observed at the 6:15 P. M. count, due probably to a heavy kill of hornflies by the 5:00 P. M. spraying, since these flies were brought into the barn with the cows. The picture was very similar for the August tests.

The trend was quite different for the stablefly (Fig. 2). The infestation increased rapidly from hour to hour up to 11:00 A. M. on both sprayed and unsprayed cows. From 1:00 P. M. to 3:00 P. M. there was a marked decrease in the number of stableflies on the cows. Following the 3:00 P. M. count the infestation increased on the check animals, reaching its peak for the day at 7:15 P. M. Similar trends were observed in the August spraying.

COMPARATIVE ABUNDANCE OF THE SPECIES.—During both tests there were more hornflies than stableflies on the cows except after 3:30 P. M. during the first test, when stableflies were either as numerous or more abundant. Hornflies were more numerous in July than in August except after 2:00 P. M. when the infestation was about the same. The stablefly population was much lower during August than it was during July.

CONCLUSIONS.—An oil base cattle spray with 6 per cent toxicants including pyrethrum and two grades of a thiocyanate (*Lethane*) applied to cows at rates of 0.5 cc., 1 cc. and 2 cc. per 3.23 sq. ft. of body surface area was repellent to the hornfly for a period up to 10.5 hours after spraying. Similar application rates were less repellent to the stablefly both as to amount and duration. Little repellency against this species was observed after four to five hours following spraying. As the time interval following spraying increased, the amount of repellency to both species decreased. In parallel tests, 2 cc. of spray per 3.23 sq. ft. of surface area was more repellent to the hornfly than 0.5 cc. for 7 hours after the morning sprayings. At other times no significant differences were noted. The heavier application was more repellent to the stablefly only for 4 hours after morning spraying, and for 2 hours after the evening spraying. The hornfly infestation was greater in the morning than during the afternoon, but the stable-fly infestation was greatest in the evening.—7-11-44.

Observations on the Biology of the Stablefly in Florida

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During investigations on the breeding places (Simmons & Dove 1941 and 1942) and control (Dove & Simmons 1942) of the stablefly, or "dog fly," *Stomoxys calcitrans* (L.), in northwestern Florida in 1939–42, certain interesting biological facts were obtained. In this article are presented data obtained in studies of infestations in bay grasses, peanut litter, waste celery, and a prepared laboratory medium.

THE EGGS.—The eggs of the stablefly are deposited promiscuously in favorable breeding media. Groups of many eggs may be concentrated into small areas, or the eggs may be scattered individually throughout the media. They are not deposited in clumps, cemented together as are the eggs of the blowfly, nor are they cemented to the medium, although the mucoid covering may adhere lightly to the substratum. Not only are eggs readily deposited on suitable fermenting vegetation, but oviposition is also heavy before fermentation begins, and flies confined without access to a suitable medium will deposit eggs at random throughout the cage.

Table 1 is a record of the minimum and maximum incubation periods and the percentage of hatch of 5969 eggs at 28° C. involving 40 separate tests, over a period of approximately 2 months. The eggs were deposited on wet black cloth, from 8 separate cages, each containing about 100 flies. The ratio of males to females in the cages was approximately equal, and in some instances exact ratios were set up at the beginning of the tests. Eggs deposited on the black cloth were counted and transferred to new cloth over wet filter paper, in a covered Petri dish, and placed in a cabinet at 28° for hatching. Observations were made to determine the initial and final hatchings in each of the 40 tests.

The minimum incubation period encountered at 28° C. was 19 hours and the maximum 120 hours, while the weighted arithmetic-mean minimum was 39.65 hours, and the mean maximum 65.1 hours. On this basis the calculated arithmetic-mean incubation period was 52.3 hours.

The highest hatch obtained was 96.9 per cent, the mean 30.45 per cent, and in a few instances hardly any hatch occurred. The mean percentage of hatch was lower than expected; however, the adult flies were confined during November and December in a laboratory where no heat was available except during part of the day. This resulted in a definite curtail-

[1] The assistance given in this work by E. B. Blakeslee and E. E. Rogers of this Bureau is gratefully acknowledged.

Table 1.—Incubation period and percentage of hatch of *Stomoxys calcitrans* eggs at 28° C., Panama City, Fla., November and December 1939.

Eggs Tested	Incubation Period Minimum	Maximum	Eggs Hatched
Number	*Hours*	*Hours*	*Per Cent*
49	43	67	28.6
254	31	65	48.4
33	41	65	12.1
78	30	30	30.7
60	24	27	50.0
311	19	72	60.8
159	31	55	21.4
174	49	56	14.4
473	49	53	3.6
148	48	120	54.1
246	48	120	5.7
189	31	96	66.1
194	24	72	3.6
90	31	72	43.3
290	49	72	59.0
478	31	48	5.9
47	72	79	4.3
172	50	72	58.7
40	48	72	30.0
64	24	72	96.9
196	48	72	5.1
179	48	54	59.2
208	48	72	12.9
59	48	72	30.5
103	48	77	11.6
84	48	72	23.8
110	24	72	37.3
136	48	96	34.6
20	48	53	10.0
95	48	72	21.0
114	48	72	57.0
174	36	60	78.7
89	29	53	17.9
294	29	53	26.2
68	48	77	4.4
110	48	77	20.9
76	24	72	3.9
128	48	96	39.1
11	48	72	36.4
166	48	55	21.7
Total—5,969	—	—	—
Arithmetic mean—	39.65	65.1	30.45[2]

[1] In a few instances initial and final hatchings occurred at night, and in such cases the minimum and maximum designations may vary slightly from the actual.
[2] Total number, 1818.

ment of activity, with a probable consequent reduction in mating, as well as in oviposition. During warm weather more normal results were obtained.

THE LARVA.—Like other muscid larvae, the stablefly has three instars. In determining the duration of these instars, newly hatched larvae were placed on Richardson's medium, as modified by Doty (1937), usually at 30° C., within 1 hour after hatching, and were observed at 2-hour intervals thereafter until they had developed to the third instar, A total of 106 larvae, the progeny of both wild and laboratory-reared flies, were examined.

First instars were present in the medium during the first 80 hours of incubation, and the earliest molt to the second instar occurred at 44 hours after hatching. In general, however, second instars were not present in appreciable numbers before 65 hours, nor after 144 hours of incubation. From 44 to 80 hours after hatching, therefore, both first and second instars were present. Third instars were found after 97 hours of incubation, which constitutes an overlapping of second and third instars from the 97th to 144th hours. In summation, therefore, first instars were found from the 1st to the 80th hours, second instars from the 44th to 144th hours, and third instars from the 97th hour to pupation, which may begin as early as the 148th hour. In 23 separate tests conducted from April to June 1940, and involving as many individual cages of flies, the mean combined minimum egg-larval period was 165.8 hours, or 6.9 days.

During November and December 1939, 6 separate tests, involving a total of 806 newly hatched larvae, were conducted by transferring the specimens to Richardson's medium and incubating at 28° C. The minimum larval period in each test was 7 days, but in 27 tests conducted in January, February, and March 1940, at 30° C. the mean minimum egg-larval period was 11.2 days, or 3.5 days longer than was required for similar development during May and June 1940. The longer developmental period encountered during midwinter, even at summer temperatures, suggests that the cause may be the physiological phenomenon of a heterodynamic diapause.

Larval development in a suitable bay-grass medium was equally as rapid as in Richardson's medium. On May 18, 1940, about 1,000 newly hatched larvae were placed in a battery jar containing 1 gallon of turtle grass, *Thalassia testudinum* Koenig and Sims, mixed with a small amount of shoalweed, *Halodule wrightii* Aschers, which had been collected green 2 days before. This was incubated at 32° C. and within 5 days pupae were present. Under favorable conditions larval devel-

opment in bay grass in the field is equally rapid, flies having emerged from test plots within 13 days after oviposition. The maximum duration of the third larval stadium is not definitely known but may endure for months, as will be discussed later.

THE PUPA.—During the period from October 1939 to June 1940, a total of 49 separate lots of pupae, the progeny of 50 individual cages of flies, were observed to determine the minimum duration of the pupal stadium. Five lots of pupae from Richardson's medium, incubated at 28° C. during October, November, and December 1939, had a mean minimum pupal period of 7 days (Table 2), whereas 21 lots incubated at 30° during January, February, and March 1940 had a mean minimum period of 7.4 days, and 23 lots incubated at 32° during April, May, and June 1940 had a mean minimum period of 5.8 days. The pupal period, like the larval, was longest in midwinter and shortest in the spring, although the incubation temperature was 2° higher during the latter tests. The mean minimum pupal period for the 49 lots of pupae under observation was 6.55 days. The maximum larval period is largely dependent on the temperature, environment, and availability of food.

The percentage of survival of pupae or larvae varies considerably, as is shown by the fact that of 208 first instars implanted into Richardson's medium in January 1940, only 82 flies, or 39.4 per cent, emerged; whereas in another test involving 163 first instars, 132 flies, or 80.9 per cent, emerged.

THE ADULT.—In northwestern Florida adult stableflies are usually present throughout the winter and are active during the warmer periods. In 1940, an unusually severe winter, no adults were seen in nature from January 19 to March 10. This period of inactivity was initiated by a temperature of 16.5° F. with a mean maximum of 57.8°. Flies captured on March 10, however, deposited fertile eggs within 24 hours but lived only 1 week in the laboratory. Flies were not absent at any time during the winters of 1941–43, when the temperatures were more nearly normal.

Under favorable conditions adult flies may emerge within 13 days after oviposition, but the greater number usually emerge during the 14th to 18th days. In these studies adults were fed citrated beef blood supplied twice daily in pipettes, similar to the method described by Bruce & Eagleson (1938). The blood was usually warmed to 37.5° C. before it was used, but flies will feed readily on blood no warmer than room temperature. In some instances blood was supplied to flies in a membranous sac of processed sheep intestine suspended in the cage. Flies readily punctured the membrane and fed, and in a few instances took rather old and stale blood.

PREOVIPOSITION PERIOD.—In the laboratory, at room temperatures, during April, May, and June 1940, the mean minimum preoviposition period, based on 23 individual cages of flies, was 10.6 days. During January, February, and March, however, the mean preoviposition period was 13.2 days, but the environmental temperature was lower than that encountered during the spring months.

Table 2.—Minimum pupal period for 49 lots of pupae incubated at 28°, 30°, and 32° C., respectively, during the period October 1939 to June 1940 at Panama City, Fla.

Minimum Pupal Period When Temperature Was—				
28° C. (Oct. to Dec. 1939	30° C. (Jan. to March 1940)		32° C. (April to June 1940)	
Cage No.	*Cage No.*	*Days*	*Cage No.*	*Days*
26 & 27	60	8	101	8
30	62	8	102	7
49	64	9	103	6
50	66	8	104	5
51	67	7	105	5
	68	8	106	6
All 7 days	69	8	107	7
	70	7	108	6
	71	6	109	6
	72	6	110	5
	73	6	111	6
	74	6	112	5
	76	8	113	6
	79	7	114	6
	80	7	115	6
	81	8	117	6
	83	7	118	5
	84	7	D8	6
	85	7	D9	5
	86	7	D12	6
	87	7	D13	5
			D14	6
			D15	5
Means	—	7.4	—	5.8

Table 3.—Record of laboratory-reared flies which emerged December 5, 1939, and were kept at room temperatures, Panama City, Fla.

Dates	Flies in Cage	Eggs Laid	Incubation Period		Eggs Hatched
			Minimum	Maximum	
1939:		*Number*	*Hours*	*Hours*	*Per Cent*
Dec. 14	30	114	48	72	57.0
Dec. 15	28	174	36	60	78.7
Dec. 16	—	89	29	53	17.9
Dec. 17	—	294	29	53	26.2
Dec. 18	18	68	48	77	4.4
Dec. 19	—	110	48	77	20.9
Dec. 20	13	78	24	72	3.9
Dec. 21	—	2	—	—	0
Dec. 23	12	128	48	96	39.1
Dec. 24	—	11	48	72	36.3
Dec. 25	4	166	48	55	21.7
Dec. 27	3	0	—	—	—
1940:					
Jan. 1		12	—	—	0
Jan. 2	0	0	—	—	—
Total	—	1244	—	—	—
Means	—	—	40.6	68.7	33.28

Eight days is the shortest preoviposition period encountered, and this occurred only twice in 20 tests, during the spring of 1940, and in each instance flies were fed citrated beef blood, usually twice daily, during the preoviposition period.

ADULT LIFE SPAN.—The maximum adult life span encountered in the laboratory was 47 days, in a study of about 200 flies captured from a steer on November 17, 1939. Obviously, the total maximum life period was more than the 47 days recorded, since it included age prior to capture, which in some instances was at least 8 days, as fertile eggs were laid 2 days after the flies were captured, and the mean minimum preoviposition period was 10.6 days. Only 43 per cent of the flies in this cage were dead on the 18th day following capture, but the mortality rate increased sharply after this time. A total of only 4000 eggs were deposited during the period of survival, but the flies were kept in a room heated only part of the day, which greatly curtained activity. Eggs deposited during the last week of survival were often infertile and some were only collapsed shells.

The mean maximum laboratory life period of 20 cages of adults collected during the period of September 22 to December 15 was 15.8 days. During January, February, and March 1940, 23 cages of flies whose larvae were reared on Richardson's medium had an average maximum life period of 18.3 days, whereas 22 cages kept during April, May, and June 1940 had an average maximum life period of 17.4 days. The maximum survival period of flies in nature is not definitely known, but Bishopp (1939) says investigators have kept adults alive under abnormal conditions for more than 3 months, and Knowlton & Rowe (1934) say that adults may live for more than 2 months, with an average life of approximately 3 weeks. Table 3 is a record of a representative colony of laboratory-reared flies. Undoubtedly the number of eggs laid by these flies and the percentage of hatch were adversely influenced by the low room temperatures encountered.

RATE OF ENGORGEMENT.—Table 4 is a combined record of 2 series of tests on the time required by the stablefly for engorgement when feeding on goats at room temperature. Individual flies were captured from cages, in shell vials, and applied directly to the skin of the goat, where they fed readily. Both feeding and observation were facilitated by previously clipping the hair from the area. Of 14 feedings on host No. 1, a brown female goat, there were 8 complete engorgements in an average period of 4 minutes and 52 seconds. On host No. 2, a white female goat, there were 12 engorgements out of 14 feeding attempts, with an average period of 5 minutes and 3 seconds. The mean engorgement period was 4 minutes and 59 seconds, the minimum period recorded was 1½ minutes, and the maximum where constant feeding from a source of blood was maintained was 8 minutes. Of the 28 attempts at feeding, 16 were made with female flies, 11 with males, and in 1 instance the sex was not recorded. Fifteen of the females completed engorgement, the average period being 5 minutes and 30 seconds, whereas only 5 of the males engorged, but the mean feeding time was 3 minutes and 24 seconds. These tests show, therefore, that female flies fed more readily than did the males, but required about 2 minutes longer to engorge. In 8 instances the flies ceased feeding before attaining engorgement, but in at least 1 instance constant feeding was maintained for 10 minutes without marked success of engorgement. Prolonged unsuccessful attempts at engorgement were frequent, and it is significant that flies often punctured the skin in 4 or 5 separate places in close proximity before striking vessels where blood could be obtained. This factor increases the injury and annoy-

Table 4.—Time required for engorgement by *Stomoxys calcitrans* feeding on goats, Panama City, Fla., February-March 1940.

Date (1940)	Duration of Feeding				Extent of Engorgement from—		Age of Fly From—		Sex of Fly From—	
	Host No. 1		Host No. 2		Host No. 1	Host No. 2	Host No. 1	Host No. 2	Host No. 1	Host No. 2
	Min.	*Sec.*	*Min.*	*Sec.*			*Days*	*Days*		
Feb. 20	1	30	2	15	Partial	Full	12	12	Male	Male
Feb. 22	—	—	5	25	—	Full	—	15	—	Female
Feb. 23	5	23	—	—	Full	—	15	—	Male	—
Feb. 26	5	0	6	51	Full	Full	6	19	Female	Female
Feb. 28	3	0	8	0	Partial	Full	8	12	Male	Female
March 1	5	10	2	15	Full	Full	1½	1½	Female	Female
March 4	3	7	10	0	Partial	Partial	6	18	Male	Male
March 6	8	0	7	0	Full	Full	8	8	Female	Female
March 8	5	0	5	20	Full	Full	9	9	Female	Female
March 11	7	3	5	0	Partial	Full	5	?	Female	Female
March 12	2	10	3	0	Full	Full	6	6	Male	Male
March 13	5	0	4	15	Partial	Partial	7	7	?	Male
March 13	5	21	5	20	Partial	Full	7	7	Male	Female
March 13	4	0	3	10	Full	Full	7	7	Female	Female
March 13	4	12	7	3	Full	Full	7	7	Male	Female

ance caused to both man and animals by the feeding of stableflies, and enhances the possibility of mechanical transmission of certain blood-borne diseases. If, however, a fly strikes a good blood supply on the first attempt, engorgement is rapid and the annoyance consequently reduced.

The average age of flies used in the tests was 8.6 days, the minimum age 36 hours, and the maximum 19 days. Full engorgement was accomplished by flies of both the minimum and maximum ages.

BITING RATES AT SPECIFIED HOURS.—Tests conducted at Sarasota, Fla., in May 1942 showed that *Stomoxys* flies fed more readily early in the morning and late in the afternoon, and became sluggish during the heat of midday. The range of temperature during the tests was from 70° to 89° F.

Flies were captured for the tests by placing screen cages 6 by 6 by 3 feet over heavily infested celery litter. After a few days several thousand adults were in the cages and usually fed readily when given access to a host. Man was used as a host in all instances, and was exposed to bites intermittently from 7 a.m. to 7 p.m. over a period of about 1 week. In making exposures the host entered the cage and the arms or legs, or both, were exposed for bites. Table 5 shows the biting rates of caged *Stomoxys* at various times of the day.

BREEDING INCIDENCE IN BAY-GRASS MEDIA OF DIFFERENT AGES.—Dog flies will oviposit on green bay grass as soon as it is deposited on shore by the tide and will continue to do so for some time afterwards, but the grass is much less favorable for fly development if oviposition does not take place until after it is a week old. This was demonstrated by isolating several lots of green uninfested bay grass under fly-tight wire-mesh cages, and subsequently exposing them to oviposition at various intervals. Each lot of bay grass was approximately 2.5 feet in diameter and 16 inches high and consisted of a mixture of *Thalassia testudinum* and *Halodule wrightii*. Natural beach exposures were made after 7, 14, 21, 28, 35, 39, 49, 56, 63, and 70 days. Fourteen days after exposure the

Table 5.—Rates of biting of caged *Stomoxys calcitrans* on limbs of human hosts at specified hours, Sarasota, Fla., May 1942.

Time of Day	Total Exposure	Total Bites	Biting Rate per Minute
	Minutes	*Number*	
7 a.m. to 10 a.m.	38	139	3.65
10 a.m. to 4 p.m.	50	85	1.70
4 p.m. to 7 p.m.	133	317	2.38
Average biting rate per minute between 7 a.m. and 7 p.m.	—	—	2.44

Table 6.—Emergence of dog flies from bay grass exposed to natural infestation for 14 days after being isolated for various periods, Florida, 1942.

Locality	Grass		Number of Flies Emerging from Bay Grass Isolated for (Days)										
	Species	Per Cent	0	7	14	21	28	35	39	49	56	63	70
Fort Walton	*T. testudinum*	90	50	6	17	13	0	1	0	2	0	0	0
	H. wrightii	10											
Panama City	*T. testudinum*	50	230	47	17	0	0	0	0	0	0	0	0
	H. wrightii	50											
Apalachicola	*T. testudinum*	50	187	104	59	2	0	2	0	0	0	0	0
	H. wrightii	50											
Total	—		467	157	93	15	0	3	0	2	0	0	0

grass was again caged and kept under observation for about 30 days, during which time emerging flies were counted daily. The results of the exposures on the various lots of grasses are summarized in table 6.

On the average, less than half as many flies emerged from grass isolated for 7 days as from grass exposed to oviposition immediately after it was washed ashore. Grass initially exposed after 14 days' isolation reared only 19.91 per cent as many flies as grass exposed while fresh. After 21 days' isolation emergence dropped to 3.21 per cent, and grass isolated longer than this produced hardly any flies. Freshly-deposited green grass exposed at intervals during the tests readily became heavily infested.

The significance of these tests lies in their practical application in control. Grass deposited on shore is treated with a spray consisting of creosote and bay water, both to prevent breeding and to eliminate existing infestations. Obviously it would not be economically feasible to treat uninfested grass after it had been on shore for as long as 3 weeks, and even after 2 weeks the practicability of treatment is questionable. If grass becomes infested shortly after it is deposited, however, it may contain larvae and pupae for several weeks and must be treated to eliminate this infestation, even though it is probably not capable of breeding a new generation of flies.

OVERWINTERING.—A favorite overwintering place for stableflies in northwestern Florida is peanut-litter piles left in the field after harvesting (Simmons & Dove 1941). A less important place in this vicinity is manure mixed with grain or hay about dairy and stock barns. The principal overwintering form appears to be the third instar. Pupae and adults are able to survive over much of the season.

Peanut litter usually becomes heavily infested soon after the middle of October. In many instances shallow piles of litter become unfit for breeding after a month or two, but late infestations may linger on in the third instar during the cool weather and gradually transform into pupae. Emergence occurs on warm days during the winter, but as late as March larvae may be found in litter unfit for fresh breeding. This shows that after the onset of cold weather the further development of third instars is greatly attenuated until the advent of warmer weather. when development is completed and flies emerge. Peanut litter exposed after cold weather begins may not become appreciably infested, but litter exposed earlier in the fall is infested with third instars until spring, indicating survival throughout the winter.

In one overwintering test 100 pupae, 100 third instars, and 145 first instars were placed in a medium of old, nonnutritious bay grass for overwintering tests. They were subjected to outside temperatures; which in the case of the pupae and third, instars reached a minimum of 15° F. These temperatures were far lower than are usually encountered in this area, and the specimens were transferred from an incubator to a subfreezing temperature in a medium having no heat of fermentation or nutritional value. None of the specimens survived. One hundred pupae placed in

Spanish moss also failed to survive. If the third instars had been in a medium suitable for food, such as peanut litter, some survival might have been expected.

SUMMARY.—Results of tests and observations on the development and feeding habits of the various stages of *Stomoxys calcitrans* L. are given. The minimum incubation period of the eggs tested at 28° C. was 19 hours and the maximum 120 hours. The mean minimum was 39.65 hours, the mean maximum 65.1 hours, and the calculated arithmetic-mean incubation period 52.3 hours.

In Richardson's medium, at 30° C., first instars were found from the 1st to the 80th hours, second instars from the 44th to 144th hours, and third instars from the 97th hour to pupation. The developmental period was longer during midwinter, even when rearing was carried on at summer temperatures. In a fresh bay-grass medium, development was equally as rapid as in Richardson's medium.

The mean minimum pupal period of 49 lots of pupae was 6.55 days at temperatures ranging from 28° to 32° C., and the longest period was encountered during midwinter. During April, May and June the mean minimum pupal period was only 5.8 days.

Adult flies captured in nature and fed on citrated beef blood lived as long as 47 days in captivity, but most of them died before this time. The minimum preoviposition period encountered was 8 days, and the mean of 23 lots was 10.6 days.

Feeding stableflies often punctured the skin of their host several times before drawing blood, but when blood was found engorgement was accomplished in 1½ to 8 minutes, with a mean of 4 minutes and 58 seconds. Tests at Sarasota, Fla., showed that *Stomoxys* under semi-field conditions fed on human hosts more readily early in the morning and late in the afternoon, and became sluggish during the heat of midday.

Bay grass isolated for 7 days before flies were permitted to oviposit on it produced less than half as many adult flies as grass infested while green. Grass initially exposed to flies after 14 days' isolation produced only 19.91 per cent as many flies as grass exposed while green. After 21 days' isolation emergence dropped to 3.21 per cent. Uninfested grass as old as 21 days need not be treated to prevent subsequent fly breeding.

Third instars of *Stomoxys* overwinter in peanut litter in northwestern Florida. Slow development occurs throughout the winter and great numbers of flies emerge during warm periods.

The cycle from egg to adult may be as short as 13 days or may, under adverse temperature conditions, require several months.—4-22-44.

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Phony-peach quarantines of Georgia, Illinois, Missouri, South Carolina, Tennessee, and Texas have been modified to apply to 1944 uniform areas. Peach mosaic quarantines of Arizona and Texas have been so modified.

Screwworm Survey of the Southeastern States in 1943[1]

W. G. Bruce, *U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine*

The screwworm, *Cochliomyia americana* C. & P., was abundant and destructive in the southeastern states in 1943, in evidently the most serious outbreak since 1935, especially in peninsular Florida and southern Georgia. Losses of livestock due to screwworm attack were heavy and in some Florida counties exceeded those of any previous year.

The mild winter of 1942–43 permitted unabated screwworm activity throughout southern Florida, and climatic conditions during the spring permitted a rapid build-up and dissemination of the insect throughout peninsular Florida and extending into southern Georgia. Other factors contributing to this outbreak of screwworms include the shortage of experienced farm labor, unavailability of effective screwworm remedies, and the failure of some stockmen to follow the approved livestock practices which prevent screwworm attack.

Wartime demands for manpower deprived the stockmen of many experienced "cowhands" and forced them to employ inexperienced help, and frequently even that was not available. Consequently much of the livestock was seldom, if ever, examined for wounds or screwworm infestations, and hundreds of cases were never treated. The fact remains, however, that a majority of screwworm infestations result from man-made wounds or wounds that can be prevented or protected by farmers or stockmen.

The unavailability of effective screwworm remedies and preventives was due in part to the fact that several of the chemicals used in approved remedies are critical war materials. Stockmen were attempting to treat screwworm infestations with all sorts of materials such as phenol, creosote, undiluted stock dip, and various proprietary remedies. Many of these materials aggravated rather than promoted wound healing and resulted in repeated reinfestations.

The Bureau of Entomology and Plant Quarantine initiated and cooperated with State agencies in a screwworm survey of the southeastern states, begun in June 1943. The purpose of this survey was to determine the extent and relative abundance of screwworm infestations and to ascertain the amounts of critical insecticides needed for screwworm control. It served the further purpose, however, of acquainting stockmen with Smear 62, the most effective screwworm remedy and preventive.

The incidence and relative abundance of screwworms in the Southeastern States were determined by contacting 10 or more farmers and stockmen in each county, representing ownership of approximately 15 to 20 per cent of all livestock in that county.

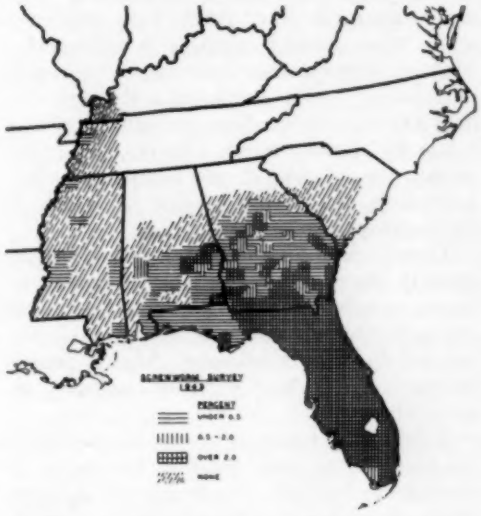

Fig. 1. Screwworm survey of the southeastern states in 1943.

The incidence and relative abundance of screwworms in the southeastern states are shown in the accompanying map (Fig. 1). The shaded counties indicate the counties surveyed, and the various types of shading indicate the degree of infestation in each county. For simplicity of interpretation the degrees of infestation are indicated as light, medium, and heavy. Infestations of 0.5 per cent or less are considered light infestations and apply to those counties having only a few scattered cases. Infestations of 0.5 to 2.0 per

[1] Acknowledgment is made of the assistance of A. L. Smith and C. C. Skipper of the Bureau of Entomology and Plant Quarantine in gathering information for this article.

cent are represented as medium infestations, and infestations of over 2.0 per cent are indicated as heavy infestations.

FLORIDA.—A heavy population of screwworms had built up in peninsular Florida before the initation of the survey in June. Southern Florida is ordinarily the exclusive overwintering area for screwworms for the entire Southeast. Information gathered from stockmen, county agents, veterinarians, and other interested parties indicated that screwworm activity began unusually early in the spring, and this was followed by a rapid build-up of large screwworm populations and their spread to adjacent areas, aided by favorable climatic conditions and large numbers of untreated infestations.

The spring and summer of 1943 were unusually dry. Scant precipitation during this part of the year is favorable to screwworm development in that the cattle feed in the lowlands during the spring and early summer and until late summer when the upland grazing is adequate. Mature screwworms leaving the wounds and falling upon comparatively dry soil find a favorable medium for pupation and adult fly development. During the rainy season these lowlands are usually flooded, and such conditions inhibit or preclude fly development.

Livestock management in Florida has greatly improved in the last 10 years, but large numbers of cattle and hogs still roam the woods and are infrequently observed by the stockmen. Many screwworm infestations in these animals go untreated.

Effective screwworm remedies and preventives were not readily available to stockmen for the treatment of infestations. Benzol was scarce, and the supply of pine-tar oil was inadequate. Proprietary remedies were plentiful, and large quantities were being used. Many other materials such as creosote, phenol, stock dip, axle grease, and various home-made concoctions were being used. Smear No 62 was practically unknown there.

The screwworm survey covered the entire State of Florida except Monroe County on its southern tip, where the livestock population is extremely light and the screwworm problem is of minor importance.

The infestation in various counties ranged from 0.5 to as high as 15.0 per cent, depending chiefly on the class of livestock and the kind of management practiced in different areas. Dairy cattle and pure-bred or high-grade animals, which are usually associated with good livestock practices, suffered much less from screwworm attack than the neglected cattle and hogs roaming the woods.

In western Florida, where infestations are predisposed mainly by shear cuts, birth of young, bites of the Gulf Coast tick, and wounds in hogs caused by fighting, the infestation was light, owing to the activity of stockmen in the timely application of preventives to open wounds.

Approximately 99 per cent of the causes predisposing animals to screwworm infestations in Florida were as follows: Birth of young, Gulf Coast tick, castration, branding, earmarking, and snags and scratches. Snags, scratches, and wounds from fighting were probably the most important predisposing causes of screwworm infestations in hogs.

Screwworm infestations were reported in 100 per cent of the untreated wounds in peninsular Florida during July, August, September, and October. There was a gradual decrease during November and an estimated infestation of 35 per cent of the wounds in December. The gradual decrease in the actual number of screwworm cases was made possible by the availability in Florida of Smear 62 and by more aggressive action on the part of stockmen in the treatment of infested and uninfested wounds.

Deaths of livestock due to screwworm attack could not be accurately determined but according to reports of stockmen and county agents these losses were immense. Okeechobee County reported a loss of 1200 calves. Two stockmen in Marion County each reported a loss of at least 200 head of cattle. Similar reports were received from many other Florida counties.

GEORGIA.—Screwworm infestations were widespread in the southern three-fourths of Georgia and were particularly abundant in the extreme southern part of the state. Stockmen in these areas reported that screwworm activity in 1943 was observed approximately 6 weeks earlier than in any previous year. It is possible that some screwworms overwintered in southern Georgia, but apparently the infestations were due to the

natural migration of the screwworm fly from Florida.

Infestations ranged from 0.5 to over 5 per cent of all livestock in the infested counties. Deaths from screwworm attack were reported to be heavy in some counties, but a reasonable estimate of these losses could not be made.

The spread of screwworms in Georgia was effectively checked shortly after supplies of Smear 62 were made available and this remedy was applied to wounds.

ALABAMA.—The natural migration of screwworm flies from the western parts of Georgia and Florida extended the infestation to most of the southern third of Alabama. In general the populations were small, and a further spread of the pest was inhibited after Smear 62 was made available.

Heavy infestations in Lee, Macon, and Montgomery Counties were probably due to the shipment of large numbers of cattle from infested areas into this area for grazing. Several carloads of these cattle were shipped in from Florida.

In the center of the infested area there were three counties, Crenshaw, Conecuh, and Covington, in which no infestations were found. These counties are chiefly concerned with the growing of cotton, and susceptible animals are comparatively few.

MISSISSIPPI.—A careful survey of every county of Mississippi revealed only a few scattered screwworm infestations in 8 of the 82 counties. Infestations in Lauderdale and Clarke Counties, which border the Alabama line, were probably the result of natural migration of the screwworm fly from Alabama. Other infestations in Jackson, Yazoo, Hinds, Warren, Grenada, and DeSoto Counties were more probably due to the importation of infested livestock. This is especially likely in Yazoo County, where cattle were periodically shipped in from Texas.

A number of cases were reported along the Mississippi River which, upon investigation, were found to be infestations of blowfly maggots.

TENNESSEE.—The survey in Tennessee was limited to 18 western counties. Infestations had been reported in adjacent states—Arkansas and Missouri—hence the advisability of surveying counties bordering the Mississippi River.

Three apparently authentic infestations were reported in Dyer County, evidently due to the importation of infested livestock. One doubtful infestation was reported in each of Obion, Shelby, and Madison Counties. Other infestations were reported, but upon investigation the maggots were found to be those of blowflies.

KENTUCKY.—The survey was extended into western Kentucky because of reported screwworm infestations in the areas adjacent to Missouri and Illinois. No infestations were found in the nine counties scouted.

SOUTH CAROLINA.—Screwworm infestations in South Carolina were found in only one of the 18 counties surveyed. These infestations were in Beaufort County, in the southernmost tip of the State. One infestation was reported in each of Colleton, Orangeburg, Saluda, and McCormick Counties, but careful scouting failed to authenticate any of these reported cases.

SUMMARY.—A screwworm survey of the Southeastern States in 1943 revealed widespread infestations in Florida, Georgia, and Alabama. Infestations reached outbreak proportions in peninsular Florida and southern Georgia with a considerable loss of livestock.

Climatic conditions favorable to the screwworm, together with the shortage of experienced farm labor and the unavailability of effective remedies, contributed to the rapid build-up and spread of screwworms in the Southeast.

As a result of this survey, the incidence and relative abundance of screwworms throughout the Southeastern States were determined, and a fair estimate was obtained of needed critical insecticides. Furthermore, the survey ensured the proper distribution and conservation of these insecticides, and aided in the reduction of screwworm infestations and attendant losses.

The survey acquainted stockmen with the most effective procedure and medicine for screwworm control. In a goodly number of cases these control practices were applied and contributed to a reduction in losses and a reduction in the severity of the outbreak.—7-24-44

The Toxicity of Sulfur Dioxide, Acetone and Ethylene Oxide Alone and in Combination[1]

ELY M. SWISHER, *Ohio State University*[2]

The mixing of chemical compounds (sprays and dusts as well as fumigants) offers many new possibilities in the search for new and improved insecticides. These mixtures also present an interesting but rather complex problem to the toxicologist attempting to measure quantitatively and compare their relative toxicities and to ascertain the type of joint toxic action exhibited by a mixture. The problems of joint toxic action are not well understood and entomologists engaged in the toxicological comparison of mixtures should familiarize themselves more thoroughly with the aspects and possibilities of mixed insecticides to be able accurately to evaluate such mixtures. The present policy of loosely labeling all or nearly all promising mixtures as "synergistic" should be discarded by entomologists (and also the Patent Office) since it leads only to further misunderstanding and is certainly misleading to anyone attempting to evaluate accurately the possibilities of a mixture so loosely labeled by its sponsor.

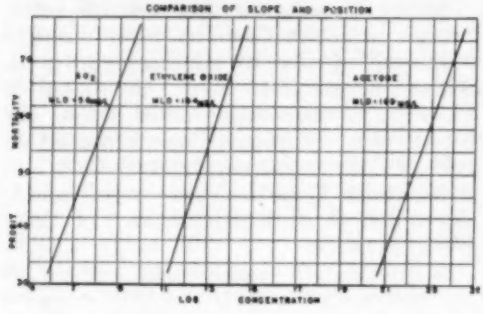

FIG. 1.—Comparison of the relative toxicity of SO_2, ethylene oxide and acetone against the confused flour beetle.

The present paper reports the results of a series of laboratory tests with mixtures of sulfur dioxide and acetone, and sulfur dioxide and ethylene oxide. These tests were designed and conducted in an attempt to obtain a clearer understanding of joint toxic action and the methods of determination. To evaluate the toxicity of a mixture it is necessary also to test the separate components of the mixture. Therefore the toxic action of sulfur dioxide, acetone, and ethylene oxide alone was studied and compared.

APPARATUS AND PROCEDURE.—The equipment used in the present work was especially designed to make accurate, rapid and comparable fumigations with gases or highly volatile liquids, or both. All fumigations were made in 12-liter round glass flasks. These flasks were placed in a constant temperature cabinet with their necks extending through the top of the cabinet, and held in place by removable wooden collars. Each flask was closed with a neoprene stopper through which was inserted three glass tubes. Tube A was used for the introduction of gases from a gas burette. Volatile liquids were introduced into the flask through tube B. After the desired dosages were introduced and thoroughly mixed, the insects were dropped through tube C and into an organdy sack tied securely to the end of the tube.

All fumigations were conducted at a temperature of 25° C. and 5 hours exposure. The confused flour beetle, *Tribolium confusum* Duval was used as the test insect and only adult beetles 3 to 5 weeks old were used. Final mortality counts were made 120 hours after exposure.

RESULTS.—Dosage-mortality curves for sulfur dioxide, acetone, and ethylene oxide alone were computed (Bliss 1935a). The relative slope and position of these curves is compared in figure 1. To the writer's knowledge, a dosage-mortality curve for acetone against the confused flour beetle (or any other insect) has never been established, probably because the use of acetone alone as a fumigant would be impractical. However, the vapors of acetone are definitely toxic, and it is slightly more toxic to *T. confusum* than carbon tetrachloride (Shepard 1939).

Observations were made of the effects of these fumigants on the test insects during and after exposure to concentrations

[1] Independent research project supervised by Professor F. L. Campbell, Dept. Zoology & Entomology, Ohio State University.

[2] Now with Rohm and Haas Co., Philadelphia.

estimated to produce approximately 80 per cent mortality. Although the dosage-mortality curves for these fumigants (Fig. 1) are nearly parallel, the post fumigation reactions of the beetles to each fumigant was markedly different (Fig. 2). Sulfur dioxide is a quick acting fumigant and only a small percentage of the beetles, seemingly dead after 5 hours exposure, recovered. Acetone exerts an anesthetic effect on the beetles. Dosages that will eventually kill approximately 10 per cent or more of the beetles will completely anesthetize 100 per cent of the individual for several hours. Ethylene oxide exhibits a delayed action effect. After 5 hours exposure all the beetles were very sluggish and an accurate distinction between dead and alive was difficult to make for several days.

Three types of joint toxic action defined by Bliss (1939) are now recognized by insect toxicologists for mixtures of insecticides. This classification is based on the relative toxicities of the components when applied singly and in the mixtures. These three classifications are *independent* joint action, *similar* joint action, and *synergistic* joint action. The statistical methods used to ascertain the types of joint toxic action exhibited by a mixture are discussed by Bliss (1939) and Finney (1942).

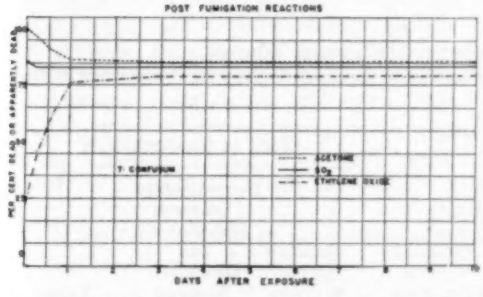

FIG. 2.—Post-fumigation reaction of the confused flour beetle to SO_2, ethylene oxide and acetone.

For the present toxicological studies of the action of SO_2-acetone and SO_2-ethylene oxide mixtures, a series of dosage-mortality curves were obtained for SO_2-acetone mixtures in the ratios of 1 part SO_2 to 10 parts acetone, by weight, 1:20, 1:30, 1:40, and 1:50, and for SO_2-ethylene oxide mixtures in the ratios of 1:1 and 1 part SO_2 to 5 parts ethylene oxide, by weight. For this study of joint toxic action, the mixtures that can be accurately tested are restricted by the ratio of toxicity of one component to the other and no effort was made to eliminate mixtures which might not be useable in practical application.

Figure 3 illustrates the dosage-mortality curves for each of these mixtures, comparing their relative slope and position. Theoretically the curves in each series should be parallel to each other in slope, and actually it can be seen that they do not depart significantly from the parallel position.

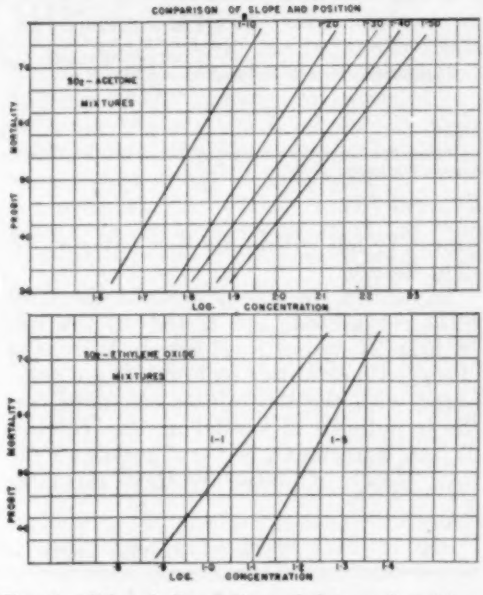

FIG. 3.—(Above) Comparison of slope and position of five SO_2-acetone ratios. (Below) Comparison of slope and position of two SO_2-ethylene oxide ratios.

The toxic action of each of these mixtures was statistically compared with the toxicity of the components applied alone, and tested to ascertain the type of joint toxic action exhibited by the mixtures, according to the methods outlined by Bliss and Finney. In table 1 is listed the experimental data for tests of five different dosages of the 1:30 ratio SO_2-acetone mixture. The mortalities *observed* for each of these dosages are listed in column 7. The mortalities *calculated* for these dosages on the hypothesis of *independent* action are listed in column 8. A chi-square test of agreement between the calculated and observed mortalities indicates that the discrepancy is highly significant and thus the toxic action of this mixture must be as-

Table 1.—Test for independent action.

1	2	3	4		5		6	7	8	9	10
No.	No. Insects	Dose of Mixture in mg/l	of Which SO_2	of Which Acetone	[1]Per Cent Kill of Each SO_2	[1]Per Cent Kill of Each Acetone	Per Cent Kill of Mixture	[2]Probit Kill of Mixture	[3]Indep't Action Probits	Indep't Action Weights	X^2 Test of Agreement
1	50	124.0	4.0	120.0	5.4	4.1	89.5	*6.2544*	*3.6763*	16.3	108.3
2	60	130.2	4.2	126.0	8.4	6.9	93.1	*6.4814*	*3.9506*	25.3	162.04
3	87	133.4	4.3	129.1	10.2	8.6	94.5	*6.5944*	*4.0808*	40.36	255.00
4	45	111.6	3.6	108.0	1.8	1.15	77.8	*5.7642*	*3.1043*	6.93	49.03
5	50	114.6	3.7	111.1	2.4	1.7	81.5	*5.8953*	*3.2562*	9.7	67.58

[1] Obtained from formulae for dosage-mortality curves for SO_2 and acetone alone.

$$Y_{so} = -3.183864 + (10.920021)\ X_{so}$$
$$Y_a = -21.12559 + (11.731420)\ X_a.$$

[2] Obtained from formula for dosage-mortality curve for the 1:30 ratio SO_2 acetone

$$Y_m = -16.169518 + (10.711642)\ X_m.$$

[3] Obtained from formula for independent action

$$P = P_1 + P_2 - P_1P_2^*.$$

$X^2 = 641.9$
with 5 degrees of freedom
(highly significant)

Where P is the proportion killed by the combination when P_1 and P_2 are the proportions killed by the same quantities of the two components applied alone.

sumed to fall within another category of joint action.

In table 2 the five dosages of the 1:30 ratio SO_2-acetone are tested on the hypothesis of *similar* joint action. When the observed mortalities (column 4) are compared with the mortalities calculated on the hypothesis of *similar* action (column 6) the agreement is very good and the toxic action exhibited by these mixtures can be assumed to fall within the concept of *similar* joint action.

Likewise the SO_2-acetone ratios of 1:50, 1:40 and 1:20, and the SO_2-ethylene oxide ratio of 1:5 were found to exhibit *similar* joint action. No definite conclusions could be made on the action of the 1:10 ratio SO_2-acetone and the 1:1 ratio SO_2-ethylene oxide because the quantities of the less toxic components present in these

Table 2.—Test for similar action, 1:30 SO_2-acetone mixture.

1	2	3	4	5	6	7	8	9
						Test of Significance		
No.	No. Insects	Dose of Mixture in mg/l	[1]Probit Kill of Mixture	[2]Equiv. Dose in mg/l	[3]Similar Action Probits	Similar Action Weights	Discrepancies between Calculated and Observed Squared	X^2 Value
1	50	124.0	*6.2544*	7.058	*6.3709*	15.6	$(.1165)^2$	.211727
2	60	130.2	*6.4814*	7.4113	*6.6093*	14.1	$(.1279)^2$	.230653
3	87	133.4	*6.5944*	7.5936	*6.7282*	17.4	$(.1338)^2$	.3115024
4	45	111.6	*5.7642*	6.3525	*5.8480*	21.96	$(.0838)^2$	.154212
5	50	114.8	*5.8958*	6.5347	*5.9939*	23.4	$(.0981)^2$	.225192

[1] Obtained from formula for the 1:30 regression line

$$Y_m = -16.169518 + (10.711642)\ X_m.$$

$X_3 = 1.13328$ with 5 degrees of freedom (not significant)

[2] Obtained by multiplying the dose by $(\pi_{so2} + \rho\pi_{acet})^*$.

[3] Obtained from formula $Y_{sim} = (a - b\bar{x}) + b_{common} \log_{equiv.\ dose}$.

If rho (ρ) represents the potency of the second component (acetone) relative to that of the first (SO_2), and if a mixture contains two components in the proportion π_1 and π_2 respectively, a given dose of the mixture, z_m, has as its *equivalent dose* of the first component the amount $(\pi_1 + \rho\pi_2)z_m$.

mixtures are entirely below the limits of their dosage-mortality curves.

DISCUSSION.—Since the present test mixtures were found to fall within the category of *similar* joint action and exhibit no evidence of synergism, no actual data were available for synergistic tests. Nevertheless, a clearer understanding of joint toxic action and the difference between *similar* joint action and *synergistic* action can be gained from a study of the present tests.

Many entomologists frequently assume that synergistic action is demonstrated by a mixture of two substances, a given dose of which produces a per cent kill greater than the additive per cent kill produced by the two components if applied alone. Apparently the U. S. Patent Office also accepts such a definition of synergism. This is a loose and incorrect application of the term since the toxic action exhibited by such a mixture may be (and evidently almost always is) in either the category of *independent* or *similar* joint action. In the above test No. 1 for the 1:30 SO_2-acetone ratio (Table 1) when a quantity of SO_2 sufficient to kill 5.4 per cent of the test insects (*Tribolium confusum*) is mixed with a quantity of acetone sufficient to kill 4.1 per cent the mortality produced by the mixture is 89.5 per cent. This difference in mortality seems at first glance to be greater than would possibly be expected from such a combination and thus be a demonstration or example of *synergistic* action. However, by a closer examination *similar* action is actually demonstrated. In a mixture of two substances which in combination exhibit *similar* joint toxic action, one component can be substituted at a constant proportion for the other if their relative proportions in the mixture are known. In the 1:30 SO_2-acetone ratio it has been shown that when the acetone in the mixture is theoretically replaced by an equivalent toxic dose of SO_2 the predicted kill (column 6) is in close agreement with that actually observed for that dose of the mixture (column 4, table 2). The range of difference in the kill produced by the components in combination and alone in the case at hand (*i.e.*, 89.5 per cent for the mixture and 5.4 per cent and 4.1 per cent for the components alone) is probably due to the relatively *high* toxicity of the SO_2 and the relatively *low* toxicity of the acetone. Mixtures of two substances more nearly similar in their relative toxicities would probably not exhibit such a range of difference.

Combinations which might exhibit *similar* or *independent* action rather than *synergism* probably offer many more practical possibilities than synergistic combinations since it seems evident that true synergism is rare. The possibilities are probably unlimited for improving insecticides through combination of two or more substances. The manufacturer or consumer may not be at all concerned with whether or not a given mixture exhibits *independent*, *similar*, or *synergistic* action as long as the product is satisfactory and presents an improvement over the use of the components alone. To the toxicologist attempting to accurately evaluate a mixture, the type of toxic action exhibited is a significant factor and the present policy of loosely labeling any and all promising combinations as "synergistic" should be discontinued.—6-22-44.

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Sulfur Dioxide-Acetone as a Household Fumigant[1]

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Many entomologists and pest control operators believe sulfur dioxide is only slightly toxic to household insects because the burning of sulfur has so often failed to give satisfactory control. The fault, however, lies not in its toxicity (Shepard, 1939) but in the method of producing the gas. New fumigants will probably replace HCN eventually but there should remain a place for SO_2, especially in the fumigation of places where bedbugs are most likely to occur and where a safe, inexpensive, effective fumigant is needed. The promotion of such uses for SO_2 depends on the development of a simple, reliable, and safe method of application.

The product obtained by mixing SO_2 with acetone has been assumed to be a solution. However Albertson (unpublished data) has recently demonstrated that a definite but readily decomposable compound, $C_3H_6O \cdot SO_2$, is formed consisting of 1 mole of SO_2 and 1 mole of acetone, and is a liquid unchanged in appearance from the acetone. The vapor pressure of this compound is such that the liquid can easily be confined, e.g. in tin cans. It is stable when confined but progressively volatile and readily decomposable into its component parts when exposed to air at standard conditions. This compound might be packaged in simple, inexpensive, nonreturnable containers and be applied for the evolution of SO_2 in household fumigation.[3]

This paper reports a study of SO_2-acetone mixtures and includes two phases of fumigation research. (1) The flammability of SO_2-acetone-air mixtures, and (2) chamber fumigations with SO_2-acetone mixtures.

FLAMMABILITY TESTS.—One of the most important physical properties of a fumigant is its range of flammability in mixtures with air. SO_2 gas is nonflammable in all proportions in air. Acetone is highly flammable and dangerously explosive in concentrations ranging from approximately 3 per cent to 8.8 per cent volume in air. Various ratios of SO_2-acetone can be expected to range from high flammability to nonflammability depending on the ratio and the concentration in air. It was considered essential in this study to ascertain the exact range of flammability for SO_2-acetone-air mixtures and especially to determine the explosive range for the molecular combination of SO_2-acetone.

The flammability of a gas in admixture with air is defined in terms of its range of flammability or explosive range. The explosive range refers to the definite limitation of combustibility and rate of burning of flammable vapors mixed with air.

APPARATUS AND PROCEDURE.—It is possible to determine the limits of flammability of a combustible fumigant in a rather simple apparatus usually called an explosion tube. The explosion tube used for the present tests was a modification of that described by Jones (1933) and the details of procedure were similar. The tube was partially evacuated through the stopcock. SO_2 gas and acetone vapor were introduced into the tube through a second stopcock. The gases were mixed by means of a paper deflector attached to a strip of metal and pulled up and down within the tube by an electro-magnet. When the gases were thoroughly mixed, the flame from an alcohol lamp was passed across the lower open end of the tube. If the gases ignited and the flame *propagated* to the top of the tube, the mixture was considered flammable. If the flame failed to propagate up the tube, or the gases failed entirely to ignite, the mixture was considered nonflammable.

RESULTS.—The curve defined from flammability tests with SO_2-acetone-air mixtures is presented in figure 1. The x's along the curve represent mixtures in which the flame failed to propagate more than half the distance to the top of the tube, and the circles denote mixtures which were found to be flammable. The lower curve represents the per cent by volume of the gases in air necessary to produce approximately 95 per cent kill of the test insect, *Tribolium confusum* Duval, with a 5-hour exposure period under laboratory conditions.

Acetone alone is dangerously flamma-

[1] Independent research project supervised by Professor F. L. Campbell, Department of Zoology & Entomology, Ohio State Univ.

[2] Now with Rohm and Haas Company.

[3] Patent pending.

ble, although compared with many flammable compounds its *range* of flammability is not great. Below the limit of 3 per cent no propagation occurred and usually these mixtures failed entirely to ignite. Mixtures above the upper limit of 8.8 per cent always ignited and burned at the source of ignition for a few seconds but failed to propagate.

The lowest ratio in which SO_2 and acetone can be combined to produce a mixture nonflammable in all concentrations in air was found to be 1.9 parts SO_2 to 1 part acetone by volume (or 34.5 per cent acetone). The molecular combination (1.6 parts acetone to 1 part SO_2 by volume) was found to have a narrow range of flammability extending from 6.5 per cent to 10.4 per cent volume in air. The concentration of this ratio necessary for a 95 per cent mortality of the flour beetle is approximately 0.6 per cent volume in air. Mixtures containing more than 75 per cent acetone by volume would be dangerous to use under practical conditions.

The molecular combination (the compound $C_3H_6O \cdot SO_2$) will not support a flame and it can be poured or sprayed directly into a flame without igniting.

It should be recalled that several of the common fumigants, *e.g.*, HCN and methyl bromide, which for all practical fumigation purposes are nonflammable, nevertheless have a definite explosive area within certain concentrations considerably above those needed for insect control. Methyl bromide is used frequently in fire extinguishers in Europe but it actually is explosive within a restricted range of 13.5 per cent to 14.5 per cent volume in air (Jones 1928). HCN has a rather wide range of flammability extending from 5.6 per cent to 40.0 per cent volume in air (Crosby-Fisk-Forester). From the practical point of view the entire range of flammability of a fumigant is not particularly important, it is the *lower limit* of the range that is the limiting factor. The lower limit of flammability for the molecular combination of SO_2-acetone is higher than the lower limit for HCN. The molecular ratio of SO_2-acetone can be considered a safe fumigant in concentrations up to approximately 8.5 lbs. per 1000 cubic feet. Such concentrations are greatly in excess of those needed for satisfactory control of household pests.

FUMIGATION TESTS.—Flammability is the factor limiting in the use of SO_2-acetone ratios containing an excess of acetone. Vapor pressure is the factor limiting ratios containing an excess of SO_2. Two ratios, the molecular weight combination and the ratio of equal parts by volume SO_2-acetone can be handled very satisfactorily and safely as liquids. Ratios containing SO_2 in excess of 50 per cent by volume are too volatile for satisfactory handling.

APPARATUS AND PROCEDURE.—A number of fumigation tests were made with

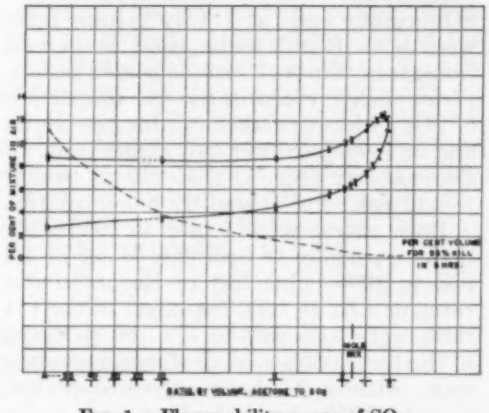

FIG. 1.—Flammability range of SO_2-acetone mixtures.

these two ratios to determine their toxicity to various household insects, the speed of aeration after exposure, and the effect of the fumigant on various materials commonly present in houses. These tests were made in a fumigation chamber of 740 cubic feet capacity. The chamber was furnished as a bedroom with a day bed, dresser, hanging clothes, various metals, and several strips of wall paper. The chamber was equipped with an exhaust fan which at full speed removed 4 volumes of air per minute, or at half speed 2 volumes per minute. The temperature was held constant at approximately 77° F., but the humidity, dosages, and length of exposure were varied. The confused flour beetle, adults and larvae of the black carpet beetle, and all stages of the bedbug were used as the test insects. These insects were placed in pillboxes and distributed about the room in both exposed and protected places.

The liquid fumigants were applied by a method developed by Glass (1944). The

desired quantity of the liquid was poured into a jar containing approximately an equal weight of *Johns-Manville Celite Catalyst Carrier, Type VI.* This is diatomaceous earth in the form of small pellets about one-eighth inch in diameter and very absorbent. The cap of the jar was then screwed tightly in place and the contents shaken until the liquid was well distributed and absorbed by the pellets. The gas was released in the chamber by scattering the pellets over a framed screen supported on a tripod, and the room then quickly closed for the period of exposure.

RESULTS.—The molecular weight ratio-SO_2-acetone and the 1:1 by volume ratio were both found to be good household fumigants. The 1:1 ratio is somewhat superior since it contains a larger proportion of SO_2. It has a smaller volume per pound and an even narrower and higher range of flammability (Fig. 1) than the molecular weight ratio but is slightly more volatile.

Both ratios were found to be highly toxic, especially to bedbugs. Doses of 1.2 lbs. to 1000 cubic feet of the molecular ratio, or 1 lb. to 1000 cubic feet of the 1:1 ratio were sufficient to kill 100 per cent of the test bedbugs of all stages, including eggs, with a 15 hour exposure period. Gough (1940) found bedbug eggs to be about four times more resistant to SO_2 than adults in laboratory tests using an exposure period of 2.5 hours.

The flour beetle and carpet beetle adults were found to be somewhat more resistant, requiring approximately 1.5 lbs. to 1000 cubic feet of the molecular ratio or 1.2 lbs to 1000 cubic feet of the 1:1 ratio with a 15 hour exposure period. Carpet beetle larvae were most resistant, requiring approximately twice the doses needed for effective bedbug control. The high resistance of carpet beetle larvae has been noted previously; *e.g.*, Gross (1938) found that this insect requires approximately twice the dose of methyl bromide needed for bedbugs and roaches.

Blickle (1942) has shown that a high initial concentration of SO_2 is desirable. SO_2 is a quick acting insecticide and when the full dose is released at once, as in the present case, a long exposure period is not necessary. Kills obtained from doses of 1:1 by volume ratio SO_2-acetone at the rate of 2 lbs. per 1000 cubic feet with 8 hours exposure were almost identical to kills obtained with 15 hours exposure. The length of exposure seems to be a more important factor when smaller doses are used.

SO_2 is highly volatile and is rapid in its penetration. This same property also tends to make the gas comparatively easy to expel from the fumigated room. Because of the strong distinctive odor of SO_2, the presence of the gas can easily be detected. All odor of the gas could be expelled from the chamber, and the materials within the chamber, in from 4 to 8 hours, apparently depending on atmospheric conditions.

SO_2 has fallen somewhat into disrepute as a household fumigant partly because under certain conditions it may damage household articles. Corrosion of metals and fading of fabrics and wall paper were observed in tests using large doses of SO_2 *alone* under conditions of very high humidity (75 per cent and up) and an exposure period of 20 hours or more. When SO_2 is applied in mixture with acetone the danger must be slight because no injury of any kind could be detected on any of the wall paper samples, colored clothes, painted surfaces, or metals even after repeated tests on the same samples over a period of several months. No injury occurred even in tests with high dosages, 15 hours exposure, and humidities of 70 to 85 per cent, and with some of the wall paper samples and colored woolens thoroughly wet with water just before exposure.

The use of SO_2 in combination with acetone further decreases the possibility of damage since other impurities present when sulfur is burned on the job are not present in the liquid mixture, and the fire hazard created by burning sulfur is eliminated. There is also evidence that the presence of acetone vapors in the air reduces the danger of injury by SO_2.

CONCLUSIONS.—From these experiments testing the physical and insecticidal properties of the molecular weight ratio SO_2-acetone and the 1:1 by volume ratio SO_2-acetone, the following conclusions can be made. (1) The application of SO_2 in mixture with acetone as a household fumigant offers a unique method of easily, safely and quickly applying the gas. (2) The molecular ratio and the 1:1 by volume ratio SO_2-acetone are nonflammable in concentrations that would be used for insect control. (3) Both ratios can be handled safely and conveniently as liquids

and could be packaged and marketed in inexpensive, non-returnable containers for use as a household fumigant. (4) Both ratios are highly toxic to household insects. (5) SO_2 is a quick acting fumigant and when the full dose is released at once a long exposure period is not necessary. (6) Doses of 1 lb. to 1000 cubic feet of the 1:1 ratio SO_2-acetone are effective with 15 hours exposure against all stages of the bedbug. Doses of 2 lb. to 1000 cubic feet of the 1:1 ratio or 2.25 lbs. of the molecular ratio with only 8 hours exposure are highly effective against carpet beetle larvae, bedbugs, and the confused flour beetle. (7) Aeration of the room after fumigation with SO_2-acetone is rapid. (8) SO_2-acetone seems entirely safe and noninjurious to household furnishings, although as a precaution fumigations should be conducted under conditions of average humidity.

SUMMARY.—Sulfur dioxide has fallen somewhat into disrepute as a household fumigant. The fault, however, lies mainly in the method of application and actually the material possesses many desirable qualities. It forms a loose chemical combination with acetone. This compound is a liquid that is stable when confined but readily decomposes into its component parts upon exposure to air at standard conditions. The molecular weight ratio of SO_2-acetone and the 1:1 by volume ratio SO_2-acetone are conveniently handled as liquids and are less flammable than HCN.

These mixtures of SO_2-acetone could be packaged conveniently (*e.g.*, in tin can containers) and be applied for the evolution of SO_2 in household and other fumigations. This new method of application greatly reduces or eliminates most of the objectionable qualities of SO_2. It outmodes the practices of generating the gas on the job, or the use of heavy, returnable steel cylinders and should promote the use of the compound especially in dwellings where bedbugs are most likely to occur and where a safe, inexpensive and effective fumigant is needed.—6-22-44.

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WHITMARSH RESEARCH LABORATORIES

The new research laboratories of the Pennsylvania Salt Manufacturing Company, established in Whitmarsh Hall, a historic mansion of nineteenth century Philadelphia, were formally opened on October 4, 1944. Ceremonies appropriate to the occasion were held and visitors were given opportunity to inspect the facilities of the laboratories. We may feel assured that the profession of entomology, as well as many other sciences, will benefit by the operation of these new and modern laboratories and we must confess a slight twinge of jealousy of those who are to be privileged to conduct their work in such sumptuous surroundings.

Effect of *Empoasca solana* on Sugarbeets Grown for Seed

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Work by the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, on insects affecting sugarbeets grown for seed began in 1938 in the beet-seed-producing areas of southern Arizona. Cage studies made during 1938 and 1939 (Hills, 1941), showed that *Lygus* spp. and certain of the stinkbugs were responsible for a reduction in the percentage of viable seed produced.

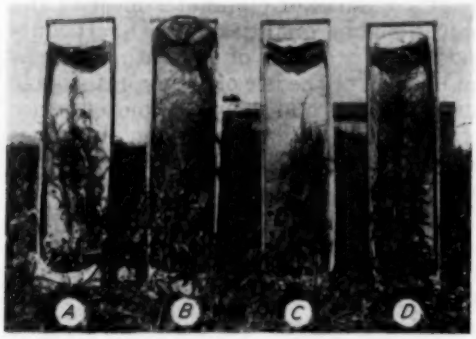

FIG. 1.—Cages on the U. S. 33 variety of seed beets 1938–39. One side of the cages has been cut away to show the plants within. Leafhoppers were introduced into cages *A* and *C;* cages *B* and *D* were maintained free from the leafhoppers. Photographed in June 1939 just before harvest.

These studies also showed that the green peach aphid, *Myzus persicae* (Sulz.), was primarily responsible for a reduction in yield, and that the false chinch bug, *Nysius ericae* (Schill.), in numbers up to 500 per plant had no measurable effect on the crop.

Studies of insect populations in the seed-beet fields were also begun during the spring of 1938, and these, up to January 1944, are considered in this paper. These studies showed that a small green leafhopper, *Empoasca solana* DeL., was the most numerous insect occurring throughout the season. These insects migrate into the fields in late fall and early winter. Breeding occurs in the beet fields, but numbers are reduced somewhat during January and February. In late March and early April the numbers begin to increase, owing to more extensive reproduction and probably additional influxes into the fields. From then on until the seed begins to mature in June, populations of this insect become high. Studies in cages to determine the type and extent of injury by this insect were conducted during the seasons of 1938–39, 1940–41, and 1942–43.

DESCRIPTION OF CAGES AND ARRANGEMENT OF EXPERIMENTS.—The experiments of 1938–39 were of a preliminary nature. Eight cages, each enclosing 1 foot of beet row, were used. They were cloth-covered, about 2 feet in diameter and about 7 feet in height (Fig. 1). Essentially, these cages consisted of a cheesecloth tube supported between side stakes and held extended by means of wire hoops. The bottom edges of the cloth were tacked onto wooden frames which were set into the soil around each 1 foot of beet row to be enclosed. Two varieties of beets were used—a curly top-resistant variety known as U. S. 33 and another variety known as U. S. 217. Four cages were placed on each variety, and leafhoppers were introduced into two of the four cages in each case.

The experiments of 1940–41 were much more detailed than those of 1938–39. Twenty-four cages were used, and these were arranged in a randomized-block experiment of four experimental treatments and six replicates. The cages were covered with a tightly woven cheesecloth. The framework of each cage consisted of two inverted, U-shaped iron rods, the lower ends of which were securely fastened into the four corners of a wooden frame. The rods were of sufficient length to make the cage 6 feet tall at the highest point. The base frame measured 24 by 30 by 8 inches, and was set approximately 4 inches into the soil. The cheesecloth was tailored to fit the frame formed by the rods and was held in place at the bottom by a lath strip nailed around the base. A slide fastener placed in the front of the cage provided sufficient opening for observations and introductions of insects. Ladybeetles were introduced into all cages to prevent aphid infestations, it having been previously determined that these insects would not attack adults or nymphs of *Empoasca solana*.

The experiments in 1942–43 were designed to give additional data necessary to

clear up questionable points of the 1940–41 experiments. The cages were similar to those used in 1940–41, but they were set up in randomized blocks of 2 experimental treatments and 20 replicates, involving 40 cages. Figure 2 shows the 1932–43 cages as they appeared 1 month before harvest.

EXPERIMENT IN 1938–39.—The 8 cages used in this experiment were set up early in December. On December 12, 1938, 1000 of *Empoasca solana* were introduced into each of 4 of the cages, and on January 18, 1939, another 1000 of *E. solana* were introduced into each of the same 4 cages, making a total of 2000 insects introduced per 1 foot of beet row. Although it was recognized that the numbers of *E. solana* introduced into each cage were far in excess of populations of this insect occurring under field conditions, it was desired in these preliminary tests to obtain indicative information regarding the economic status of this insect in its association with sugar beets grown for seed. No leafhoppers were introduced into the other 4 cages these being left as checks.

During June the seed from each cage was harvested and analyzed separately to determine the weight, volume, and percentage viable. Beet armyworms, *Laphygma exigua* (Hbn.), gained entrance into three out of the four cages on the U. S. 217 variety, reducing the yield to such an extent that the results from these four cages have not been considered with the other data. The plants in the four cages on the U. S. 33 variety were in good condition, but there was a striking difference in the appearance of the plants in the infested cages as compared with the insect free cages. Plants injured by the insects were dwarfed and produced little or no seed. Figure 1 shows the plants within the four cages on the U. S. 33 beets just before harvest. The number of plants producing seed, the yield, and the percentage of viable seed produced are given in table 1.

The methods employed in obtaining data on the weight and volume of the seed[1] produced, as well as on the percentage of viable seed by the "crack test," have been described in a previous publication (Hills 1941). The data in table 1, although meager, indicate that it is possible for very large numbers of *Empoasca solana* to reduce the yield of beet seed, but that they do not affect the viability of the seed produced or the bolting tendency of the plant.

FIG. 2.—Aerial view of 1942–43 cage set-up. The 6 cylindrical cages in the foreground were for other experiments. Photographed on May 131, approximately 1 month from harvest.

EXPERIMENT IN 1940–41.—As previously stated, this experiment involved 24 cages arranged in randomized blocks of 4 treatments and 6 replicates. The beets used were of the U. S. 22 curly top-resistant variety. Each cage enclosed 2 feet of beet row, and this 2-foot strip of row was thinned to include exactly 16 beet plants in each cage. Each block consisted of (1) one cage containing 100 *Empoasca*

[1] Sugar beet "seed" in commercial usage refers to the "seed ball," which may contain one or more true seeds. In this paper the common usage of referring to the seed ball as "seed" is followed.

Table 1.—Comparative data from cages containing *Empoasca solana* and insect-free cages on seed beets, Phoenix, Ariz., 1938–39.

TREATMENT	PLANTS DEVELOPING SEED STALKS	WEIGHT OF SEED, GRAMS	VOLUME OF SEED, CC.	PER CENT VIABLE SEED (CRACK TEST)
A[1]—2000 *E. solana*	5	0	0	—
B—Insect free	9	253.4	1425.0	99.0
C—2000 *E. solana*	9	77.9	464.0	92.7
D—Insect free	2	147.9	814.0	99.7

[1] These letters correspond to the *A*, *B*, *C*, and *D* of figure 1.

solana, (2) one cage containing 200 *E. solana*, (3) one cage containing 300 *E. solana* and (4) one cage maintained insect free as a check. Insect introductions were made November 28 and 29, 1940. The 100 and 200 introductions were based on numbers found to occur in the field, and even the 100 leafhoppers (50 per foot of row) were considered a high population. The introduction of 400, therefore, was definitely an exaggeration of any population found to occur in the beet fields.

The cages were examined at about weekly intervals throughout the time they were in the field. No differences in the appearance of the plants in the infested cages from those in the insect-free cages could be detected at any time. In early March a few of the insect free cages were found to contain small numbers of *Empoasca solana*, and these were sprayed with a water-soluble pyrethrum extract which satisfactorily controlled these insects. Early in May, aphids began to develop in some of the cages. Ladybeetles were introduced to control the aphids, but in spite of introductions of large numbers of ladybeetles the aphids became so numerous in two cages within the same block that a very small quantity of seed was produced, and consequently that block was discarded, leaving only five replicates in place of the six originally set up.

A short time before harvest an estimate was made of the numbers of *Empoasca solana* within each cage, to obtain some idea of the increase that had occurred from the original introductions and thus gain a better idea of the number of insects to which the plants within each cage had been exposed. These population estimates were obtained by shaking the plants and causing the insects to congregate at the top of the cage. A light metal frame enclosing approximately 1 square foot was then laid on top of the cage in such a way that the shadow could be seen on the inside. All the insects within that square-foot area were then counted and the total cage population was estimated from the number of square feet of cage surface as densely occupied by the insects. These population estimates are given in table 2.

It will be noted that the ratio of reproduction to the number introduced was not constant, but decreased with the high numbers introduced. The ratio of reproduction for introductions of 100 was about 10 to 1, for introductions of 200 it became about 6 to 1, and for introductions of 400 about 4 to 1. Just what factors were responsible for this decrease in the reproductive ratio when larger numbers were introduced is not known, but the result had a tendency to lessen any differences that might develop owing to the different numbers of insects introduced.

Upon maturity of the seed the stalks within each cage were cut and placed in separate bags. A record was kept of the number of stalks produced in each cage. These data are also given in table 2 and show that there were no differences as between treatments in the number of seed stalks produced per cage. This would indicate, as did the experiment of 1938–39, that these insects do not affect the bolting tendency of the plants.

After the seed stalks had dried, the seed from each cage was threshed and cleaned separately, and yield data were recorded. The viability of the seed was determined

Table 2.—Comparative data from cages containing varying numbers of *Empoasca solana* on seed beets, Phoenix, Ariz., 1940–41. (Five cages, with 16 plants per cage.)

Treatment	*Empoasca* Introduced per Cage	Estimated *Empoasca* Recovered per Cage	Stalks Produced per per Cage	Seed Analysis—Means Per Cage			
				Weight of Cleanout,[1] Grams	Weight of Seed,[1] Grams	Per Cent Cleanout	Per Cent Viable[2]
Empoasca	100	992	8.8	283.6	98.7	77.3	76.2
Empoasca	200	1220	9.2	259.4	73.9	75.4	75.1
Empoasca	400	1720	10.8	390.0	109.4	79.1	77.9
Insect free	0	41	10.2	365.2	131.8	74.1	81.6

[1] Difference between results for treatments *A*, *B*, *C*, and *D* not significant by the F test.
[2] By germination test.

by germination tests made between moist blotters according to the rules and recommendations for testing seed established by the Department of Agriculture (anonymous 1938). A record of the amount of "clean-out" was also kept in each case to determine whether the insects were having any effect on the plant growth. Any additional leafiness or a "witches-broom" type of growth which might occur on the seed spike because of the feeding of the insects should cause a greater amount of trash in the seed and would therefore cause a larger amount of clean-out. Both the actual weight of the clean-out and the ratio of clean-out to clean seed are given in table 2, together with the weight of the seed and results of the germination tests. Although the infested plants tended to yield considerably less seed than the uninfested plants, the analysis of variance showed that the differences in yield were not of mathematical significance. The experiment was repeated the next year with sufficient replicates to reduce the experimental error to a more satisfactory level.

EXPERIMENT IN 1942–43.—In this experiment only 2 treatments were used, but they were replicated 20 times. The cages were set up in randomized blocks in the fall on the U. S. 22 variety of sugar beets, the same as were used in 1940–41. Each cage was placed over 2 feet of beet row, but the plants were thinned to include only 10 beets per cage in place of 16 as used in 1940–41. Insect introductions were made on December 1 and 2, 150 *Empoasca solana* being placed in 1 cage in each block, the other being maintained insect free as a check. The cages were examined from time to time throughout the winter and spring, and no differences in the appearance of the plants could be noted between cages containing *E. solana* and insect-free cages.

Ladybeetles were introduced early in the spring to prevent aphid infestations but, even so, aphids became numerous in a few of the cages and it became necessary to discard 4 of the blocks. *Lygus* bugs gained entrance into the insect-free check cage of block 2, and therefore this block was also discarded, reducing the number of replicates to 15 at the end of the experiment. A few individuals of *Empoasca solana* appeared in some of the check cages, but these were controlled by spraying with a water-soluble pyrethrum extract as in the 1940–41 experiment.

No special estimates of insect populations within the cages were made in the spring as in 1940–41, but on June 16 it was noted that *Empoasca solana* leafhoppers were numerous in every cage in which they had been introduced the preceding fall. Judging from the 1940–41 data and the appearance of the insects in the cages in June 1943, approximately 1500 *E. solana* adults were present per cage at that time.

Upon maturity the seed from each cage was harvested, threshed, and cleaned separately, as in the 1940–41 experiment, except that in the 1942–43 experiment no record of clean-out was kept, and the seed were divided into large and small seed in the cleaning process. Those seed retained by an eight sixty-fourths inch screen, were termed large seed. Those passing through an eight sixty-fourths inch screen and retained by a seven sixty-fourths-inch screen, were termed small seed. Germination tests were made as in 1940–41 to determine the percentage of viable seed, and the seed was weighed to determine the yield. All data have been analyzed by the analysis of variance, and the differences required for significance at odds of 19 to 1 are given in table 3.

Table 3.—Comparative data from cages containing *Empoasca solana* and insect-free cages on seed beets, Phoenix, Ariz., 1942–43. (Fifteen cages with 10 beet plants per cage.)

Treatment	Seed Stalks Per Cage	Seed Yield (Grams Per Cage)		Per Cent Seed Germinating	
		Large	Small	Large	Small
150 *Empoasca* in cage	6.7	119.0	25.8	94.5	84.6
Insect free	7.1	339.6	51.1	95.5	85.6
Difference Required for Significance ($P=0.05$)	[1]	63.6	17.0	[1]	[1]

[1] Not significant by the F test.

The data in table 3 show that there were no differences between treatments in the viability of either large or small seed, which indicates that *Empoasca solana* did not affect the viability of the seed and this is in agreement with the experiment of 1940–41 as well as that of 1938–39. The data also show that there were no

differences due to treatment in the number of seed stalks produced per cage, which is also in agreement with past experments and indicates that these insects have no effect on the bolting tendency of the plants. Yield data in table 3 show very definite decreases in the yield of both large and small seed from cages containing *E. solana*. Furthermore, these data show that the percentage of small seed produced was greater from cages containing *E. solana* than from the insect-free cages. These data indicate that this insect is capable of reducing the total yield of seed and that it has a tendency to cause the plants to produce significantly higher proportions of small seed. The seed from the 1938–39 and 1940–41 experiments were not separated into large and small seed, but the experiments of 1938–39 showed a definite decrease in total seed yield where *E. solana* was present. A tendency toward a decrease in yield was also shown in the 1940–41 experiment, but differences were not significant at odds of 19 to 1, probably largely because of insufficient replications.

SUMMARY AND CONCLUSIONS.—The production of sugarbeet seed in outdoor cages at Phoenix, Ariz., was materially reduced by infestations of *Empoasca solana*. There also seemed to be a tendency for this insect to cause a slight increase in the proportion of small seed, but in no case was there any appreciable reduction in the viability of the seed produced on infested plants. These facts, together with observations of the plants involved and observations of the habitat of the insects, both in the field and in the cages, lead to the conclusion that this leafhopper feeds largely on the vegetative parts of the plants and that the damage attributable to this insect is largely in the nature of devitalization of the plants. In no instance has there been any evidence of a diseased condition or toxic effect attributable to this insect.—4-8-44.

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Susceptibility of Japanese Beetle Larvae to *Bacillus popilliae*

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The susceptibility of Japanese beetle larvae to the spores of *Bacillus popilliae* Dutky has an important bearing on the results to be expected from the artificial dissemination of spores of this bacterium. Dutky (1940) has described this organism and the symptoms of milky disease it causes in the host, and White (1940, 1941) and White & Dutky (1940, 1942) have reported on the field use of the spores as a means of Japanese beetle control. Dutky (1941a) has discussed the susceptibility of other scarabaeid larvae to the disease. The present paper reports results of laboratory experiments on the numerical relationships of the bacterial spores and the incidence of disease among the Japanese beetle larvae.

In the production of spores for use in dust form as practiced by the Bureau of Entomology and Plant Quarantine and cooperating state agencies, beetle grubs are inoculated with a dose of one million spores. A high incidence of disease results and the milky grubs are processed into dust form for field distribution. Not all grubs become diseased, however, upon injection of the inoculum. In view of the fact that the haemolymph of the grub into which the spores are injected constitutes the medium supporting the bacterial growth, any failure of the disease to develop indicates a lack of viability of the spores inoculated, a lack of susceptibility to the disease on the part of the grub, or an interaction between the two. The development of the disease follows a characteristic course, and with rare exceptions the result of inoculation is either a complete development of the diseased condi-

tion, in which the milky grub contains one or more billion spores suspended in its haemolymph, or a complete failure of the disease to develop at all. Thus the incidence of disease can be measured in terms of its presence or absence, and any differences in extent of development can be traced to temperature conditions and time of incubation.

To determine the effect of spore dose on the incidence of disease, various known doses of spores of *Bacillus popilliae* were injected into the body cavities of Japanese beetle larvae. Third instar grubs which had been held in cold storage were used for the experiments, but prior to most tests the grubs were incubated at a temperature of 78° F. for two weeks to insure only healthy grubs being used. Any natural disease among the grubs will show up under such conditions, and the diseased individuals, if any, can be rejected. Further checks were made by maintaining uninjected grubs under the same conditions as the injected grubs, but the natural incidence of disease among the grubs used in these tests was so low as to be negligible.

METHOD OF INOCULATION.—In preparing the spore suspensions, diseased grubs were immersed in water heated to approximately 135° F. to prevent coagulation of the blood. The blood was then drawn from the grubs and diluted with distilled water. Spore counts were made using the usual blood-cell counting techniques, and dilutions of the desired concentrations were further made to one-half, one-fourth, one-eighth, and one-sixteenth. Due to the rapid settling rates of the spores, the suspensions were thoroughly agitated by means of an electric stirrer at the time the samples were taken for counting and when the dilutions were made.

Inoculations were made by means of a microinjection apparatus consisting of a clamp holding a 2 cc. hypodermic syringe with a 30 gauge needle. A micrometer screw aligned with the plunger of the syringe regulated the volume of fluid ejected from the end of the needle. The volume ejected varied from .0035 ml. to .0041 ml., depending upon the syringe used, each of which had to be calibrated. In any one test syringes were used which varied as little as possible, but corrections for any differences were made in calculating the spore dose. Five syringes were used, each containing a different spore concentration. To better provide a random sampling of grubs, ten grubs were inoculated with one dose, syringes were changed and another ten inoculated, etc. The sequence of doses injected was chosen at random, and the syringes were well agitated when changed to reduce the error caused by the settling of spores. In most tests a total of forty grubs was injected at each dosage level. Essentially, this number represents four replicates of ten grubs each, although the spore suspensions were not replicated. The grubs were placed in individual compartments containing one cubic inch of sterilized soil to which was added grass and clover seed to provide food. After incubating the grubs for two weeks at a temperature of 78° F., the incidence of disease was determined by blood (haemolymph) examination of the living grubs. There is usually a certain amount of natural mortality independent of the diseased condition.

RELATION OF SPORE DOSE, INJECTED PARENTERALLY, TO INFECTION.—Nine separate determinations were made, using spore doses per grub ranging from about 1,000 to about 78,000. The incidence of disease resulting from the inoculations bore an obvious relation to the number of spores injected so that when the per cent of disease in probits was plotted against the logarithm of the dose, a rectilinear regression was obtained. Each curve was computed with the use of corrected probits and weights as described by Bliss (1935), and in only one of the nine tests was the variation great enough to give a chi-square large enough to make the rectilinear relationship questionable. Moreover, the slopes of the regression lines were parallel within the limits of error, although the positions of the lines varied. For descriptive purposes and to make use of all determinations, the individual curves for each test were shifted horizontally on the x axis until their mean probits fell along a single line described by the combined slope of $b_c = 1.341$ and passing through the mean probit of all series, $y = 4.863$ and the mean log-concentration of all series, $x = 3.946$. The log-concentrations, thus adjusted, were then converted to antilogarithms and plotted on a logarithmic scale against the per cent mortality on a probit scale as shown in figure 1. This method of calculation (illustrated by

Moore & Bliss, 1942) has the effect of assigning equal potencies to the spores used in the separate tests, equal susceptibilities among the nine groups of grubs, and tends to erase the errors arising from the spore counting technique. Whether or not differential potencies among the spores and differential susceptibilities among the grubs

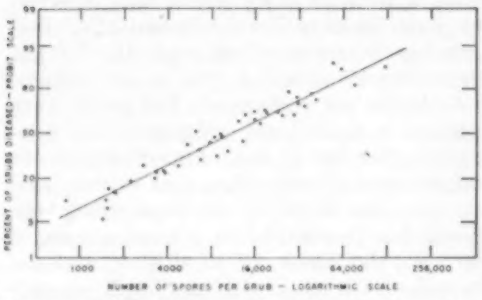

FIG. 1.—Relation of spore dose, injected parenterally, to rate of infection.

affect the slope as well as the position of the regression line is not at present known, but since from figure 1 no consistent nonlinear trend is apparent, at least it may be concluded that the incidence of milky disease is a probit response to the logarithm of the concentration of spores injected parenterally into Japanese beetle grubs.

This graded response to the dosage of spores is a population phenomenon. For the individual grub, the disease follows a characteristic course of development, or it fails to become established. There is no reason why dilution, *per se*, should affect the potency of spores, for although this technique is subject to errors (as discussed by Cochran, 1938), the proportion of viable bacteria should not be affected by the degree of dilution. It must be considered that the haemolymph varies among grubs either in the absence of factors necessary for the development of the bacteria or the presence of antibiotic factors and that the individual spores vary in their abilities to overcome such resistance to their development. The end result is that the probability of any one grub becoming infected increases with the increased spore dose.

When spores are introduced into the haemolymph of the grub they at once come in contact with the medium which supports their growth. The natural mode of infection, on the other hand, is by the ingestion of spores, either as a result of biting a diseased grub or by swallowing contaminated soil particles and food materials. This means that the possibility of acquiring the disease is lessened by several factors. The grubs may escape exposure by ingesting soil and food particles not actually containing spores. Moreover, even if spores are taken into the alimentary tract they may pass on through, ungerminated, to be discharged with the fecal matter. The manner in which the spores or the vegetative forms pass from the alimentary tract into the body cavity is not known, but there would seem to be this mechanical barrier unless there are some secretory or other factors which aid the bacteria in reaching the haemolymph.

RELATION OF SPORE CONTENT OF SOIL TO INFECTION.—The incidence of disease relative to the number of spores present in the soil was determined in the following manner. Known numbers of spores, based on the counting methods described above, were added to given weights of sterilized soil and thoroughly mixed by means of a small barrel-type mixer. In each test five concentrations of spores were used, one determined by count and dilutions of this of one-half, one-fourth, one-eighth, and one-sixteenth. In the calculations the concentration was expressed as the number of spores per gram, dry weight, of soil. Grubs placed in individual compartments were provided with this soil, to which was added grass and clover seed for food. Each compartment was one cubic inch in size, and under the conditions of these experiments contained approximately 12 grams of dry soil. (The actual moisture content approximated 15 per cent.) This meant that with the concentration of spores used each grub was exposed to about 2,400,000 spores at the lowest dilution and almost 40,000,000 at the highest. The incidence of disease was determined by blood-examination after incubating the grubs for 17 days at a temperature of 78° F.

Three separate tests were made, and in each case, as with the parenteral injections, the probit of the response bore a rectilinear relationship to the logarithm of the spore dose. The data were calculated as before and adjusted to one curve having the formula of $y=1.761x-6.144$. The adjusted log-concentrations converted to antilogarithms and plotted on a logarithmic scale against the per cent mortality on a probit scale are shown in

figure 2. No consistent non-linear trend is evident.

Without knowing how much soil was ingested by the grubs there is no way of translating the illustrated data into terms of spore doses actually taken in by the grubs. It becomes obvious, however, that when the spores are thus in contact with the soil, very large numbers are required to cause an appreciable rate of infection.

Dutky (1941b) observed that soil containing spores at the rate of two billion per kilogram of soil should cause infection in 60 per cent to 75 per cent of the grubs. Assuming a moisture content of the soil of 15 per cent, this concentration would be equivalent to about 2,350,000 spores per gram of dry soil. While this number is somewhat lower than indicated in figure 2, it is not inconsistent with the observed data, particularly since Dutky allowed for incubation of one month.

Bacillus popilliae COMPARED WITH *B. larvae*.—Woodrow (1933) tested the susceptibility of honeybee larvae to spores of *Bacillus larvae* by inoculating spores into the food contained in individual cells. His published data showed an increase in the number of infected larvae with an increase in the number of spores introduced, but when these are plotted in terms of logs and probits, an obvious curvilinear regression is observed. Woodrow concluded that this response was not due to resistance in the individual larva, but to an escape from infection by failure to ingest contaminated food during the susceptible stage of larval life. In spite of this difference in the response of honeybee larvae to foul brood and that of Japanese beetle larvae to milky disease, certain comparisons can be made. At the higher dosage levels there is little difference between the infection rates of foul brood in honeybee larvae and of milky disease in Japanese beetle larvae (when injected parenterally). However, only about 25 *B. larvae* spores per cell are required to cause foul brood in 50 per cent of the bee larvae, whereas about 11,000 *B. popilliae* spores are required to cause milky disease in 50 per cent of the beetle larvae when the spores are injected parenterally. When the beetle grubs are placed in contaminated soil, about 2,100,000 spores per gram, dry weight, of soil are required to cause 50 per cent disease. Moreover, Woodrow found that a single spore of *B. larvae* was sufficient to cause infection, whereas the probability of one spore of *B. popilliae* causing infection, even when injected parenterally, is extremely slight. From these data, it appears that *B. larvae* is far more virulent an organism against bee larvae than is *B. popilliae* against Japanese beetle larvae.

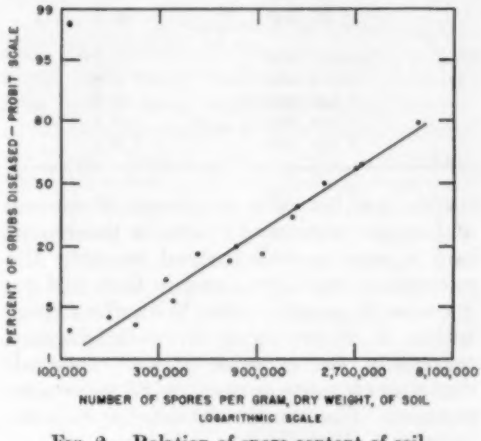

FIG. 2.—Relation of spore content of soil to rate of infection.

RELATION OF SPORE DOSE TO INFECTION.—As before mentioned, the above tests fail to show the relation of infection rate to the number of spores actually taken in the alimentary tract. Consequently, enteral injections of spores were made in a dosage series. These injections were made without apparent injury to the grub by the use of the microinjections apparatus with a blunt hypodermic needle. The grub was held in the left hand, and with fine forceps the mandibles were spread apart so as to slip the buccal cavity over the needle. The grub was pushed on the needle far enough to insure the measured dose of inoculum remaining in the alimentary tract without being regurgitated. This meant that the needle followed the lumen of the esophagus.

Thirty-five grubs were inoculated with each dose and incubated under the conditions described above. After 2 weeks the living grubs were examined for disease, results being as shown in table 1. Test A. The incidence of disease is obviously far less than when the spores are injected directly into the blood, which might be expected in view of the fact that the spores easily pass through the alimentary tract in an ungerminated condition. But also, the

Table 1.—Incidence of disease resulting from enteral injection of spores.

	Number of Spores Injected Per Grub	Per Cent of Grubs Diseased after Two Weeks
Test A	776,800	20.7
	391,400	3.2
	195,700	3.1
	92,200	0.0
	47,600	3.0
Test B	18,687,000	9.7
	9,344,000	2.9
	4,400,000	2.9
	2,318,000	3.1
	1,250,000	0.0

correlation between incidence of disease and concentration of spores in the inoculum is not so well defined as with the parenteral injections, and in fact, the regression is questionable. With the expectation of observing a more satisfactory regression, the experiment was repeated, using much more concentrated spore suspensions. The results obtained are shown in table 1. Test B.

the spores were found to be present in the mid-gut. Only occasional spores were present in the esophagus itself and in the hind-gut. In grubs fixed and dissected 18 hours after injection, the spores were concentrated in the posterior region of the hind-gut, with relatively few spores scattered throughout the remainder of the alimentary tract. After three days, few spores were present even in the hind-gut suggesting that most of the bacteria had been eliminated by fecal discharge.

Resistance of Grubs to Infection.—The failure of the disease to develop in a grub could be the result of the grub's escaping actual infection in some such manner as described by Woodrow for the honeybee exposed to foul brood. This seems unlikely when the spores are injected directly into the body cavity, but is quite possible when the mode of exposure to the disease is via the alimentary tract. On the other hand, some grubs might actually be immune to the disease. What is more probable, in the light of the dosage-response and of experiments discussed be-

Table 2.—Incidence of disease resulting from parenteral injection of spores in grubs of selected groups.

	Per Cent of Grubs Infected				Per Cent of Grubs Infected
Number of Spores Injected Per Grub	Group A Original Inoculation	Group A_1 "Resistant" Grubs of Group A	Group B Control Grubs	Number of Spores Injected Per Grub	Group C Control Grubs Fresh Spores
		Test A			
18,900	43.9	21.7	63.0	17,200	66.7
9,500	38.3	20.7	58.3	8,700	47.2
4,800	23.7	15.4	51.5	4,300	27.0
2,200	15.0	5.7	6.2	2,000	18.9
1,200	10.2	2.4	10.8	1,100	5.4
		Test B			
36,400	57.0	8.0	25.8	37,200	68.2
19,500	43.5	10.0	7.4	19,900	46.4
9,000	20.8	21.4	6.4	9,200	32.4
4,600	14.5	7.0	20.7	4,700	25.0
2,300	8.2	0.0	0.0	2,300	11.5

Again the incidence of disease is much less than would be expected, and the relationship between infection rate and spore dose is not clearly defined. It appears that with this method of inoculation, the spores are in a condition or a location unfavorable for disease development. To determine just where the spores were applied in the gut, injected grubs were fixed and dissected immediately after injection. The bulk of

low, a grub may resist infection when exposed to a given number of bacteria, but will succumb if the inoculum is increased. A preliminary experiment in which grubs failing to take the disease after one inoculation were reinoculated with higher dosages of spores indicated that the grubs were not immune, but acquired the disease in a dosage-response. The level of infection, however, was lower than following

the original inoculation, suggesting more resistance than is found in an unselected grub population. This test was repeated in a more critical way as follows.

A large number of grubs (Group A) was inoculated with milky disease spores in serial dosages and incubated for two weeks at 78° F. At the same time uninoculated grubs were maintained under the same environmental conditions. At the end of the incubation period the incidence of disease was determined, and those grubs not diseased (Group A_1) were reinoculated with spores from the same suspensions used before and which had been kept refrigerated in sealed vials. None of the uninoculated grubs showed disease, indicating no naturally acquired disease among the grubs used. These grubs were divided into two groups (B and C). One group (B) was inoculated with spores from the same suspensions used in inoculating groups A and A_1. Another group (C) was inoculated with spores freshly drawn from diseased grubs in group A. All grubs were then incubated for another two weeks at 78° F. and the incidence of disease was determined. It should be noted that groups B and C represented similar grub populations injected with different spore suspensions; groups A_1 (the "resistant" grubs of group A) and B represented different grub populations injected with the same spore suspensions; group A was injected with the same spore suspension as A_1 and B, but on a different day. The results of these tests are tabulated in table 2, Test A. The similarity in response between groups B and C demonstrates that in this case the spores lost no potency by being held for two weeks in the refrigerator. Whether the response of group A_1 should be compared with that of group A or of group B is open to question. Probably it should be compared with the response of group B, for both these injections were made on the same day. In either case, the incidence of disease in group A_1 is significantly less, indicating a definite resistance on the part of the grubs. The experiment was repeated, following the same techniques, and somewhat different results were obtained, as seen in table 2, Test B. In this case it becomes obvious from a comparison of groups A, B and C that that the spores used in inoculating A_1 and B had lost potency during the two weeks they had been refrigerated. For this reason the reduced incidence of disease noted in group A_1 can be attributed principally to a loss of potency of spores rather than to resistance on the part of the grubs. The variation in response of the grubs in groups A_1 and B is too great to allow detection of any effect of grub resistance over and above the effect of loss of spore potency.

Although this test does not confirm the first, the results of the two experiments seem sufficiently definitive to conclude that grubs may manifest an actual resistance to the milky disease, and that this resistance on the part of the grubs and reduced potency of the disease organism may act independently in causing an incidence of disease lower than expected.

A somewhat similar experiment was conducted by exposing grubs to contaminated soil rather than injecting them. As described above, known numbers of spores in serial dosages were mixed with soil, grass seed was added, and grubs were incubated in the usual manner. Control grubs, similarly incubated in sterilized soil, indicated no naturally occurring disease among those used. At the end of the incubation period the incidence of disease was determined by macroscopic examination. The soil containing each spore concentration was then thoroughly mixed and divided into two portions. In one portion were placed the grubs failing to take the disease during the first exposure, and in the other portion were placed control grubs. After incubation the incidence of disease was determined by blood examination, the results being indicated in table 3.

Table 3.—Incidence of disease resulting in grubs of selected groups reared in inoculated soil.

Number of Spores per Gram (dry weight) of Soil	Per Cent of Grubs Infected		
	Group A Original Exposure	Group A_1 "Resistant" Grubs of group A	Group B Control Grubs
3,313,700	60.0	75.8	79.2
1,642,300	38.1	62.7	58.0
811,700	19.6	37.5	32.8
403,700	6.0	23.4	15.2
199,700	3.8	4.8	10.3

It should be noted that the spore content of the soil is assumed to be the same in the three cases. It is not known whether the incubation of group A in the soil had

any effect on the spore content or not. The apparent increase in disease incidence in groups A_1 and B over group A can probably be traced to the method of disease determination. The incidence of disease in group A was determined by macroscopic examination as it was considered undesirable to wound the grubs prior to their replacement in contaminated soil. Consequently it is very probable that some of the grubs considered healthy actually harbored the disease in its early stages. On the other hand, the incidence of disease in groups A_1 and B was determined by blood examination.

When grubs are inoculated with the causal organism of milky disease, exposure to the disease can be considered uniform in point of time. In contrast, grubs placed in contaminated soil become exposed to the disease to the extent of their swallowing spores while feeding, which can neither be controlled nor satisfactorily determined as to quantity or frequency. Whether or not this tends to mask any expression of resistance is not known. In any case, this experiment fails to indicate any decrease in susceptibility among grubs having once avoided the disease.

SUMMARY.—The incidence of milky disease among third instar Japanese beetle larvae is a probit response to the logarithm of the concentration of spores of *Bacillus popilliae* injected parenterally. Similarly, when beetle grubs are reared in soil containing the disease organism the incidence of disease follows a probit response to the logarithm of the concentration of spores.

As judged by these two modes of infection the virulence of *Bacillus popilliae* in causing milky disease of Japanese beetle larvae is far less than that of *B. larvae* in causing foul brood of honeybee larvae.

Spores of *Bacillus popilliae* injected into the alimentary tract of beetle grubs do not demonstrate a well-defined dosage effect, and the incidence of disease is less than expected.

Japanese beetle grubs failing to take the disease after a parenteral injection of bacterial spores may be less susceptible to a second injection, indicating a kind of resistance. Reduced potency of bacterial spores may also act, independently, to cause less disease than expected.

In one test, grubs failing to acquire milky disease after one exposure to contaminated soil did not show any diminished susceptibility when given a second exposure. This apparent contradiction of other tests may be due to the technique employed in detecting the disease.—4-28-44.

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SCIENTIFIC NOTES

Difference in Damage by Three Species of Lygus to Alfalfa Seed Production

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The damage caused by *Lygus* spp. to the alfalfa seed crop has been investigated by Carlson (1940), Sorenson (1939), and Stitt (1940). In these investigations no note was made of the comparative degrees of injury caused by the different species. Field observations during the years 1935 to 1941, inclusive, and preliminary cage studies in 1941 by the writer indicated that the three species, *Lygus hesperus* Knight, *L. oblineatus* (Say), and *L. elisus* Van Duzee, found in the alfalfa seed fields of Arizona and southern California, do injure the seed crop to considerably different degrees.

In order to obtain more definite information on this subject, 10 replications of an experiment were made on alfalfa (variety A-136) growing in a plot on the farm of the United States Bureau of Plant Industry, Soils, and Agricultural Engineering at Bard, Calif., through the cooperation of E. G. Noble, Superintendent.

Cages were placed over alfalfa stems in full bloom just when the very small embryo seed pods were appearing. Each replicate consisted of four cheesecloth cages placed on different stems of the same plant so that any effects of plant variation would be reduced. One cage was assigned to each of the three species and to an uninfested check. One female and one male insect of each species were placed in their assigned cages selected at random in each replication. These cages were observed daily, and any dead *Lygus* were removed and replaced with live specimens of the same sex and species. These daily observations were continued for a period of 2 weeks, which corresponds approximately to the length of the main full-bloom period of seeding alfalfa.

Table 1.—Mean percentages of pods set and of brown seed of alfalfa infested by *Lygus* spp. in experimental cages, Bard, Calif., 1942.

Species in Cage	Mean Percentages of	
	Pod-Set	Brown Seed
Lygus hesperus	20.11	39.42
Lygus oblineatus	22.98	30.17
Lygus elisus	23.30	20.86
Check (uninfested)	28.40	7.33
Difference required for significance (5% level)	4.68	9.44
Difference required for high significance (1% level)	6.33	12.75

The experiment was begun on June 17, 1942, and ripe seed racemes were collected from all cages on July 21. The samples of seed racemes were examined in the laboratory for the number of pods which produced seeds (pods set), flowers which failed to produce pods (flower fall), brown seeds (nonviable), and good seeds. Percentages of pods that set and brown seeds were computed from the above data for each treatment. The records made on flower fall were used in figuring percentages of pods set.

The variation in the percentages of pods set and brown seed due to the different species of *Lygus* was analyzed by the variance method. As shown in table 1, all three species reduced pod-set significantly and caused highly significant increases in the percentage of brown seeds, as compared with the check. The decreases in mean percentage of pods set associated with the respective species are not significantly different. *L. hesperus* caused the highest percentage of brown seed. The difference between the mean percentages of brown seed caused by *L. hesperus* and *L. elisus* is highly significant, but that between the percentages caused by *L. hesperus* and *L. oblineatus* is hardly significant. *L. oblineatus* was responsible for a considerably greater percentage of brown seed than *L. elisus* although the difference did not quite reach the 5 per cent level of significance.

The results of this experiment strongly indicate that of the three species of *Lygus* present in the alfalfa seed fields of Arizona and southern California, *L. hesperus* causes the greatest damage and that *L. elisus* causes the least damager, per individual insect.—6-7-44.

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Tests of Poisoned Baits to Control the Tobacco Budworm

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The effectiveness of a number of bait mixtures in controlling the tobacco budworm, *Heliothis virescens* (F.), on tobacco have been tested. Morgan & McDonough (1917) tested arsenate of lead, antimony sulphid, Paris green, tripotassium arsenate, orthoarsenite of zinc, antimony arsenate, and antimony oxid in various strengths and in combinations with corn meal, gypsum, and fuller's earth as carriers. A mixture of 1.0 pound of arsenate of lead and 75 pounds of corn meal was found to be the most efficient combination against the budworm. Chamberlin & Garrison (1926) have shown that corn meal is preferred by the tobacco budworm even to its natural food. Chamberlin & Morrill (1936) tested zinc ortho-arsenate, calcium arsenate, manganese arsenate, magnesium arsenate, sodium fluosilicate, a sodium sulfur compound, and derris in various concentrations with maize meal as alternative insecticides for lead arsenate and maize meal in the control of the tobacco budworm. All of these mixtures, as well as others prepared with lead arsenate and various carriers, were either ineffective in controlling the larvae or caused excessive foliage injury. Cham-

berlin & Madden (1937) have reported that cubé is ineffective against the tobacco budworm. These same authors (1942) have tested the effectiveness of the standard lead arsenate-corn meal mixture (1 to 75 by weight) when prepared by six different methods of mixing. Efficient control was obtained with all of the bait-mixing methods, and chemical analyses made on duplicate samples indicated a satisfactory uniformity of mixing by the different methods.

The experimental work reported in this paper consists of laboratory tests conducted at the Tobacco Research Laboratory, Chatham, Virginia, for the determination of the relative effectiveness of five different materials in controlling the tobacco budworm. Poisoned baits prepared from *Alorco Cryloite*,[1] basic copper arsenate,[2] *Baricide*,[3] and *Dutox*,[4] each diluted with corn meal at the rate of 1 to 75, were compared with the standard lead arsenate-corn meal mixture.

Table 1.—Toxicity of five different materials to the tobacco budworm under laboratory conditions, Chatham, Va., 1943.

Treatments[1]	Number of Insects Tested	Percentage Dead at End of—		
		First Day	Second Day	Third Day
Lead arsenate—corn meal	49	32.7	77.6	93.9
Dutox—corn meal	53	41.3	52.6	66.0
Baricide—corn meal	36	2.8	5.6	5.6
Alorco Cryolite—corn meal	50	36.0	56.0	74.0
Basic copper arsenate—corn meal	49	40.8	71.4	89.8
Check	43	2.3	7.0	9.3

[1] Mixtures prepared at the rate of 1 to 75, by weight, in all cases.

PROCEDURE.—The stems of either young tobacco plants or tobacco suckers were cut the desired length, inserted through a small opening in the center of a screen wire cover, and the ends placed in water in a small, glass container. Each tobacco bud was placed separately inside a glass lantern globe with a cheese-cloth cover over the top.

The budworms to be used in the tests were collected from tobacco fields. For each test, consisting of all five treatments and one check, budworms of approximately the same instar were used. Larvae in the third, fourth, and fifth instars were used during the course of the experiment. The test was repeated more than fifty times. One-eighth of a teaspoonful of each poison was placed on separate tobacco buds, a budworm placed on each bud, and observations made at 1-, 2-, and 3-day intervals. The criterion of mortality was complete death. The record was discarded for those budworms that were killed by parasites prior to the completion of the 3-day observation period.

RESULTS AND DISCUSSION.—The experimental data are shown in table 1. The application of the Chi-square test to the results at the end of the third day from basic copper arsenate and lead arsenate showed no significant difference to exist between these two materials. A Chi-square value of 0.544 was obtained. However, a comparison of the results obtained at the end of the third day from basic copper arsenate, lead arsenate, and *Alorco Cryolite* showed the difference between these three materials to be significant. In this case a Chi-square value of 8.99 was obtained. Obviously, *Dutox* was likewise less effective than either lead arsenate or basic copper arsenate against the tobacco budworm. More deaths occurred from natural causes than from the ingestion of bait containing *Bari-cide* as the toxic ingredient.

Apparently basic copper arsenate and lead arsenate, when mixed with corn meal at the rate of 1 to 75, are equally effective in controlling the tobacco budworm. Of the materials previously tested in poisoned baits against the tobacco budworm, those that showed toxicity equal to or greater than lead arsenate were found to injure the tobacco plants. Morgan & McDonough (1917) found Paris green to be more toxic to the tobacco budworm than lead arsenate but to give plant injury under certain conditions. Chamberlin & Morrill (1936) found ortho-arsenate of zinc and calcium arsenate to give slightly better control of budworms than lead arsenate, but both materials severely injured the tobacco leaves. The low content of soluble arsenic in basic copper arsenate should make it safe for inclusion in a poisoned bait for control of the tobacco budworm.

SUMMARY.—The results of laboratory tests for the determination of the relative effectiveness of five different materials in poisoned baits against the tobacco budworm are reported. The poisoned baits were prepared by diluting each material with corn meal at the rate of 1 to 75, by weight. The results were determined from the mortality obtained at the end of the 3-day observation period. No significant difference in effectiveness was found between basic copper arsenate and lead arsenate. *Alorco Cryolite* and *Dutox* were significantly less effective than basic copper arsenate or lead arsenate. *Baricide* was entirely ineffective against the tobacco budworm.—4-21-44.

[1] Sodium fluoaluminate 85 per cent.
[2] $Cu(CuOH)AsO_4$ about 94 per cent.
[3] Barium carbonate 94 per cent.
[4] Barium fluosilicate 72 per cent plus sodium fluosilicate 8 per cent.

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Ether-Extract Content of Codling Moth Cocoons[1]

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Studies carried on at the Yakima, Wash., laboratory to develop a spray to destroy hibernating larvae of the codling moth, *Carpocapsa pomonella* (L.), on apple trees (Yothers, Carlson, & Cassil 1942, 1943; Newcomer *et al.*, 1944) indicated the desirability of some information on the chemical composition of the overwintering cocoons. A number of chemicals were found highly toxic to naked larvae, but harmless to them in their cocoons. This indicated that the cocoons were not being penetrated, or possibly that they were filtering out the toxicant. In some cases the larvae within the cocoons appeared to be well wetted with the liquid but without ill effect on them. Often mortality counts were very erratic, perhaps because poorly constructed cocoons were penetrated while better made ones were not.

In a preliminary study of the wax and fat content of codling moth cocoons, larvae that had partially spun up elsewhere were transferred to soft pine cocooning sticks and allowed to spin cocoons. For some of the larvae these spinnings were their first, while for others they were probably third or later spinnings. These cocoons contained many small particles of wood mixed with the silk, and they also contained 10 to 15 per cent of wax and fat as determined with an ether solvent in a Soxhlet extractor.

In order to obtain the percentage of wax and fat in pure spinnings, without the presence of wood particles, the following experiment was made: Several hundred larvae for each test were confined for about 30 days in a glass box containing many glass strips for them to spin on. As in the case of larvae spun up on wood, these larvae had previously spun partial cocoons. The spinnings were scraped from the glass and analyzed, the larvae and extraneous material having first been removed. Two samples were obtained in this manner, weighing 1.063 and 1.549 grams, respectively. Each lot was placed in the Soxhlet extractor with ether as a solvent. Results showed the samples to contain 22.6 and 30.8 per cent of wax and fat, respectively. It should be noted that these percentages are for pure cocoons of the codling moth larva, and that for normal cocoons the figures would probably vary greatly with the amount of extraneous material incorporated in them on the tree and in the soil.—5-20-44.

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[1] This work was a part of a project on orchard sanitation conducted at the Yakima, Wash., laboratory, E. J. Newcomer in charge.

[2] Resigned Nov. 25, 1943.

Olfactory Responses of Parasitic Hymenoptera in Relation to their Mass Production

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As Wheeler (1928) observed there is little doubt that responses to chemical substances either from a distance (odors) or on contact are "far and away the most important sensory reactions of insects." Such reactions are involved both in the *finding* and in the *recognition* of food, of the opposite sex, and of sites for the deposition of eggs. They are operative to the fullest extent under natural conditions. When insects reproduce in artificial confinement the need for utilizing the sense of smell for *finding* of their mates, food or egg deposition sites is greatly diminished. Nevertheless it has been observed that odor may be a critical factor in the mass production of certain species of parasitic Hymenoptera.

The very complex behavior patterns of the Hymenopterous insect appear to be dependent upon a highly developed olfactory sense. The parasitic Hymenoptera in their *sensitivity* to odors, if not in the *organization* of their reactions, are at least equal to the social forms. Their responses to oviposition stimuli are generally highly selective, the sites of oviposition being extremely restricted. The encyrtid *Diversinervus smithi*, for example, deposits its eggs only in the fused ganglion of the central nervous system of certain species of lecaniine scale insects (Flanders, 1939).

The chemotropic responses, whether positive or negative, are influenced by the physiological condition of the parasite. On the other hand the physiological condition of the parasite may be affected by odor of the host. Thorpe and Caudle (1938) found that in *Pimpla ruficollis* the olfactory responses of the female are correlated with the degree of ovarian development. In some species the act of mating causes a change in the oviposition response. Unmated females of *Coccophagus caridei* oviposit readily in parasitized mealy bugs but mated females oviposit only in lecaniine scale insects (Flanders 1937). The sex ratio of the progeny of a single female may be determined by the amount or quality of odor emanating from the host. If the odor is sufficient to result in the stimulation of the spermathecal gland during the deposition of an egg that egg will be fertilized and develop into a female, provided, of course, that the oviposition rate is not too high.

The entrance of the female into the zone of attraction to the host may result in the female moving along the odor gradient to its source and at the same time exserting its ovipositor and utilizing it as a probe.

This procedure may not occur under conditions of mass production where the host is abundant and of high density. Then no host odors may be so excessive and diffused that the odor gradient is obliterated. Under such conditions secondary stimuli, such as developmental condition or size of host are of most importance in oviposition.

It is well known that certain lepidopterous eggs exude an odor that is attractive to *Trichogramma* and causes it to oviposit. *Trichogramma* will oviposit in such eggs when separated from them by a layer of organdie. Salt (1935), however, concluded that the principal criterion used by *Trichogramma* in the selection of hosts was size. Certainly in Salt's experiments there was no host odor gradient present.

Either the host (*Sitotroga cerealella*), lacked an attractive odor or the cages were so charged with the odor that it had no stimulating effect, and secondary stimuli predominated.

Ullyett (1936) criticized Salt for not eliminating from the experiment the possibility of odor influence on host selection. Ullyett, however, presumed that the odor stimulus to oviposition affected the parasite to a greater extent when it was confined in a small space than when out in the open.

In small spaces odors tend to prevent oviposition for several reasons: (1) The production of excess odor bringing about diffusion and lack of gradient as noted above, (2) The stimulating odors may be imparted to inanimate objects forming false hosts, or; (3) The stimulating odor may be counteracted by the presence of more or less repellent vapors.

Destructive vapors, such as sulphur fumes if not present in lethal dosages, may permanently destroy the capacity of the parasite to respond to oviposition stimuli, as in the case of *Metaphycus helvolus* (Flanders, 1943.)

During the development of methods for the mass rearihg of *Macrocentrus ancylivorus* it was found that terpenoid substances from redwood lumber and fresh paint prevented oviposition. Whether the effect of exposure to these substances was permanent or temporary was not ascertained. The emanations from redwood have a slow lethal effect on adults of *M. ancylivorus*.

It was also noted that gaseous ammonia given off by decomposing, infested potatoes appeared to depress the oviposition activity of *Macrocentrus*. Frequent changes of air *surrounding* the production units was necessary in order to prevent the ammonia, originating in the production units in which the potatoes had reached the stage of decomposition, from entering units in which *Macrocentrus* was ovipositing.

In the study of *Trichogramma evanescens* Salt (1937) has shown that a female secretes volatile substances that tend to counteract oviposition by the same or other females. The length of time that such substances are effective is roughly proportional to the length of time that the female was present.

Another type of oviposition-inhibition by water-soluble substances was observed by DeBach (1944) during population studies using puparia of *Musca domestica* and its parasite *Mormoniella vitripennis* in an environment of barley seed. When the barley was used over a period of several months it accumulated water-soluble substances which resulted in decreased oviposition by *Mormoniella*. Oviposition returned to normal after the barley was washed.

In contaminated barley the parasites nevertheless found about half as many of the puparia that they found in clean barley and fed upon them. The fact that they fed on these hosts indicates that the parasites spent considerable time attempting to oviposit in false hosts, that is, barley seed that had become contaminated with the host odor, through contact with host puparia.

Although it is possible that *Mormoniella*, like *Trichogramma*, secretes substances which counteract oviposition, the fact that fresh puparia were used in each test and that no substance was deposited on the walls of the containers which inhibited oviposition within it indicate that no counteractive substance or odor was present. Apparently in this case the host puparia imparted an odor to certain of the barley grains each time the barley was used until so many grains were contaminated that the searching capacity of the parasite was greatly reduced.

It is apparent that odors are of paramount importance in the reproduction of parasitic insects. By the manipulation of odors the reproduction of such insects may be either stimulated or repressed.—5-15-44.

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Two Important Parasites of the Tobacco Budworm

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In the vicinity of Chatham, Virginia, there are two important parasites of the tobacco budworm, *Heliothis virescens* (F.). One is the braconid, *Cardiochiles nigriceps* (Vier.),[1] and the other is the ichneumonid, *Sagaritis provancheri* (D.T.).[2] Chamberlin & Tenhet (1926) have reported *C. nigriceps* as being the most effective among several species of parasites that attack the tobacco budworm in the tobacco-growing sections of Georgia and Florida. During July and August parasitism by this insect was found frequently to average from 50 to nearly 100 per cent. These same authors (1926) state that this parasite has also been reported from Alabama and Mississippi, and has been found as far north as Huntsville, Arkansas, and Clarksville, Tennessee. *Sagaritis provancheri* is recorded by Brimley (1938) as having been bred from the tobacco budworm near Tarboro, North Carolina, and has been reported by Wene (1942) as being an important parasite of the tobacco budworm in Pittsylvania County, Virginia. Collections made by Wene from various fields throughout the county during 1942 showed the parasitism to range from about 28 to 94 per cent. A parasitism of

[1] Identified by Mr. C. F. W. Muesebeck, Division of Insect Identification, Bureau of Entomology & Plant Quarantine, U.S.D.A.

[2] Identified by Mr. R. A. Cushman, Division of Insect Identification, Bureau of Entomology & Plant Quarantine, U.S.D.A.

60 per cent or higher occurred in most of the fields.

Since there is apparently no information on the importance in Virginia of *Cardiochiles nigriceps* as a parasite of the tobacco budworm, the author made observations during the summer of 1943 on the percentages of parasitism in the vicinity of Chatham, Virginia. During the same season further information was also obtained on the importance of *Sagaritis provancheri* as a parasite of the tobacco budworm. In the former case budworms in the fifth or early sixth instars, collected from tobacco fields, were reared in the laboratory until they either pupated or were killed by parasites. The budworms were fed on tobacco buds and had access to moist soil throughout the period of observation. The number of budworms parasitized by *S. provancheri* was determined from the cocoons of the parasite collected in the field and the parasites that emerged from budworms during the first few days after the latter had been taken into the laboratory. This procedure was permitted since only one parasite emerges from a single budworm.

RESULTS.—The data obtained on the parasitism of the tobacco budworm by *Cardiochiles nigriceps* are as follows: for three groups of collections at one location, involving from 7 to 14 budworms each and made during the period between late June and late August, the parasitism varied from 78.6 to 92.9 per cent; and for two groups of collections made at another location during August, involving 21 and 30 budworms, the parasitism was 95.2 and 90.0 per cent.

The data obtained on the parasitism of the tobacco budworm by *Sagaritis provancheri* are as follows: for three groups of collections at one location, involving from 82 to 92 budworms each and made during the period from June 24 to July 21, the parasitism varied from 30.4 to 43.0 per cent; a single collection of 28 budworms made on August 3 at a second location showed 42.9 per cent to be parasitized; and for six different collections at a third location, involving from 33 to 89 budworms each and made during the period from July 24 to August 24, the parasitism ranged from 15.2 to 26.2 per cent.

Measurements of the width of the head capsule were made on 340 budworm larvae. The application of Dyar's rule (Dyar, 1890) to the data obtained indicated the existence of six instars during the larval growth of this insect. Similar measurements made on parasitized larvae showed the tobacco budworm to be killed by the parasite *Sagaritis provancheri* usually in the second or third instar (more often the latter) but occasionally to reach the fourth instar before being killed.

OTHER HOSTS.[1]—Ten different species of insects have been recorded as hosts of *Sagaritis provancheri*. These are: the fall armyworm, *Laphygma frugiperda* (A. & S.); the beet armyworm, *Laphygma exigua* (Hbn.); the corn earworm, *Heliothis armigera* (Hbn.); *Cynthia atalanta* (L.); the zebra caterpillar, *Ceramica picta* (Harr.); the European corn borer; *Pyrausta nubilalis* (Hbn.); the Hawaiian beet webworm, *Hymenia fascialis* (Cram.); the yellow-striped armyworm, *Prodenia ornithogalli* (Guen.); *Lycophotia margaritosa* (Haw.); and the tobacco budworm, *Heliothis virescens* (F.).

The tobacco budworm is apparently the only reported host of *Cardiochiles nigriceps*.

DISCUSSION.—Both *Cardiochiles nigriceps* and *Sagaritis provancheri* are important parasites of the tobacco budworm in the vicinity of Chatham, Virginia. Although the numbers of budworms upon which data were obtained in the case of the former parasite are rather small, because of a high mortality rate during the course of rearing, the author believes the percentages of parasitism stated to be rather indicative of the actual situation. In view of the abundance of the adults of *C. nigriceps* in the fields under observation, especially during late July and August, it is not likely that many budworms escaped being parasitized. In the case of *S. provancheri* the author did not obtain percentages of parasitism during the 1943 season as high as those reported by Wene (1943) for the 1942 season; however during both seasons they were sufficiently high to constitute a major factor in the controlling of the tobacco budworm.

Other observations of the author on *Sagaritis provancheri* are in agreement with those of Wene (1943). The parasitized budworms are usually killed in the second or third instar of growth. Not more than one parasite emerges from a single budworm. Apparently there is no information as to when the budworms are first parasitized by the adults of this parasite.

To determine accurately the effectiveness of a given parasite is perhaps impossible when other parasites of the same host occur in the same locality. *Cardiochiles nigriceps* parasitizes the tobacco budworm in the first or second instar but does not usually kill the budworm until it is about two-thirds grown (Chamberlin & Tenhet, 1926). As previously stated, *Sagaritis provancheri* kills the budworm in the second or third instar of growth. The author is of the opinion that perhaps some of the young budworms that are parasitized by *C. nigriceps* may be killed by *S. provancheri* before the presence of the former is manifested. In such a case the actual percentage parasitism by *C. nigriceps* would be higher than that indicated by the rearing of budworm larvae as herein described.

SUMMARY.—Percentages of parasitism of the tobacco budworm, *Heliothis virescens* (F.), by the braconid, *Cardiochiles nigriceps* (Vier.), and the ichneumonid, *Sagaritis provancheri* (D. T.), in the vicinity of Chatham, Virginia, are presented. Parasitism by *C. nigriceps* varied from 78.6 to 95.2 per cent. From 15.2 to 43.0 per cent of the budworms were found to be killed by *S. provancheri*.

Head capsule measurements made on the budworm larvae indicated the second or third instar to be the stage of growth in which death usually occurred from paratisism by *Sagaritis provancheri*.

Ten different species of insects that have been recorded as hosts of *Sagaritis provancheri* are listed. The tobacco budworm is apparently the only reported host of *Cardiochiles nigriceps*.—4-26-44.

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A New Weed Host of the Beet Leafhopper

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U. S. D. A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine[1]

Since halogeton, *Halogeton glomeratus* (M. Bieb.) C. A. Mey. is closely related to Russian-thistle, *Salsola pestifer* A. Nels., an excellent summer host of the beet leafhopper, *Eutettix tenellus* (Bak.), studies were undertaken early in the season of 1943 to determine the suitability of this comparatively new introduced weed as a host of this leafhopper. Preliminary cage tests under both field and greenhouse conditions showed that the beet leafhopper would breed on this weed.

Halogeton, which belongs to the family Chenopodiaceae, was originally described from dry, sandy saline soil of Central Asia (Kirghiz-Sungaria).[2] This introduced weed was first recorded in the United States by Standley (1937) on the basis of specimens collected along railroad tracks at Wells, Elko County, Nev., in August 1935 by Ben Stahmann, and incorrectly determined as *Halogeton sativus* (L.) C. A. Mey. The next record was by Morton (1941) who reported it from three additional localities in Elko County and corrected the identification to *Halogeton glomeratus* (M. Bieb.) C. A. Mey. Holmgren (1942) in his flora of northeastern Nevada, states that it is spreading rapidly and was then in western Utah. A small infestation of this aggressive vagrant weed was observed by the senior author near Deaver, Big Horn County, Wyo., in August 1943, and in October of the same year it was observed west of Deaver in Park County. Holmgren (1943) writes: "In Nevada halogeton is known from Elko, White Pine, Eureka, Lander, Humboldt, and Pershing Counties; in Utah from Box Elder, Tooele, Juab, and Millard Counties . . . " and proposed halogeton as the common name. Halogeton is now known to occur in three states, and it is possible that this weed has become established in other states. Undoubtedly a detailed survey would add other areas to its known distribution in the Western States. This plant is capable of invading areas where the original plant cover has been disturbed or altered.[3] Halogeton (Fig. 1) has been observed growing in mixed stands with Russian-thistle, and smotherweed, *Bassia hyssopifolia* (Pall.) Kuntze, in several different areas. These plants are important summer hosts of the beet leafhopper. Morton (1941), writing on halogeton, states that this "new weed . . . from present indications may possibly become a pest like its relative *Salsola*." Blake[2] writes: "*Halogeton* may well become as w despread as smotherweed, a native of the Caspian region that was first collected in this country in 1919 (Fallon, Nev.) and is now abundant in many places from Washington to California," eastward to Montana and southward to northern Arizona. Holmgren (1943) writes: "Halogeton . . . can be expected to become as generally distributed as Russian-thistle . . . on our western ranges. . . . Halogeton is highly adaptable to different types of soil, being found in alkali places in bottom lands to areas well up into the aspen of our higher mountains, but reaches its best development in shadscale types where considerable amounts of alkali are present and the number of competitors much reduced."

FIG. 1.—Halogeton: Above plants photographed on July 8, 1943; Below—plants photographed on September 1, 1943.

In order to obtain definite data on the reproduction of the beet leafhopper on halogeton, preliminary cage tests were conducted. As an example, in a field test 10 female beet leafhoppers were introduced on May 18, 1943, into a cage that covered 0.625 square foot of soil surface containing halogeton and were removed on May 29. Their progenies were counted on September 27. The reproduction on this weed host during the 132-day period was 357, or at the rate of 572 leafhoppers per square foot of soil surface occupied by the weed host. In the greenhouse 50 female beet leafhoppers were introduced on June 14, 1943, into a cage that covered 6.25 square feet of soil surface containing 9 comparatively small halogeton plants. These females were removed on June 25. The progenies of these females were counted during the period September 27 to 30, and 2691 leafhoppers were removed from this cage. The reproduction in this cage for the total elapsed period of 108 days averaged 430.6 beet leafhoppers per square foot, or 299 per plant.

Population counts were made under natural conditions to obtain information regarding the suitability of halogeton in comparison with Russian-thistle as a host of the beet leafhopper. These counts

[1] The writers were assisted by F. K. Bracken, minor field aide, Bureau of Entomology and Plant Quarantine.

[2] Information furnished by S. F. Blake, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

[3] Correspondence with C. E. Fleming, Chief, Department of Range Management, University of Nevada, Reno.

were made on October 5 and 6, 1943, in the areas in northeastern Nevada and northwestern Utah where relatively pure stands of the two plants were found growing in close proximity. Quantitative samples were taken in 10 host-plant patches of each weed. The results show an average population on halogeton of 4.4 ± 0.9 beet leafhoppers per square foot of host-plant area, as compared with 4.7 ± 0.9 per square foot on Russian-thistle. Statistical analysis of the data showed no significant difference between the population found on the two weed hosts. Although the beet leafhopper has a long list of known host plants, only a very few are important as summer hosts. Russian-thistle is the most important of this group, owing to its suitability, abundance, and distribution. If halogeton spreads into important breeding areas of the beet leafhopper and becomes as abundant as Russian-thistle, and if the above comparative degree of suitability of this introduced weed host and Russian-thistle prevails throughout the leafhopper-infested area, it may become just as important a summer host as Russian-thistle.

Preliminary experiments conducted under greenhouse conditions in 1943 showed that halogeton was capable of acting as a reservoir of curly top virus.[1]

Beets and Russian-thistle are summer reservoirs of the curly top virus. According to the findings of Severin (1939) and from evidence obtained in tests conducted in Idaho, smotherweed is not a host of the curly top virus. With the spread of halogeton into new areas, an additional summer reservoir of the virus will become established.

The introduction and spread of halogeton emphasizes the fact that the beet leafhopper and curly top problem is not static, and that the host-plant complex for both leafhopper and curly top is constantly changing.

Since very little is known regarding the ability of this introduced weed to compete with or replace our native or naturalized flora, the determination of its economic importance as an aid in increasing beet leafhopper populations and curly top infection will have to wait for additional developments.

Not only is halogeton a host of the beet leafhopper and the curly top virus, but it has also been reported by Miller (1943) to be po sonous to sheep, and Holmgren (1943) credited C. E. Fleming as discovering the poisonous properties of this weed in 1942.—4-6-44.

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[1] The tested beet plants were examined for curly top symptoms by Albert M. Murphy, Bureau of Plant Industry, Soils, and Agricultural Engineering, United States Department of Agriculture.

Two Introduced Insects Attacking the Woolly Apple Aphid in California

A. E. MICHELBACHER AND A. D. BORDEN
University of California, Berkeley

In 1942, an experimental plot was established in the Watsonville area of California to test out different spray programs for the control of the woolly apple aphid on the Newtown pippin variety of apple. After a considerable search during the early winter an orchard was found in which the aphid population appeared to be satisfactory for the carrying out of such an experiment. In the check trees there was an increase in the number of aphid colonies as the season advanced. By late May more than 200 aphid colonies could be found on some of the check trees. In early June it was observed that the aphid colonies were being rather severely attacked by a number of predators and the parasite, *Aphelinus mali*. The number of aphid colonies rapidly declined and by July 7 no aphid colonies could be found on the check trees that had been most heavily infested. Aphid colonies were scarce throughout the entire orchard and because of the effective biological control no definite conclusions could be reached concerning the effectiveness of the different spray treatments.

Among the predators that were responsible for the control of the aphids were syrphid flies, lace wings, and ladybird beetles. The ladybird beetle, *Chilocorus bivulnerus* Mulsant, was present, but the species most abundant was an introduced ladybird beetle, *Exochomus quadripustulatus* (Linn.). This species was very important in reducing the aphid population and can apparently be found as adults or larvae or both in the apple orchards at all seasons of the year. *Exochomus* is an European species and was first introduced from Italy in 1915 (Smith, 1915). The insects were liberated in a citrus orchard at Fair Oaks, Sacramento County, California. The species is an important predator of armored and unarmored scales and also attacks mealybugs. Because the ladybird feeds on mealybugs it is not surprising that it should turn its attention to the woolly apple aphid.

In 1927, further shipments of *Exochomus quadripustulatus* were received from Italy. The information concerning this introduction has been furnished us by Professor H. S. Smith. The material was brought and sent in by Mr. E. W. Rust of the University of California. Colonies were propagated at Riverside and liberations were made during 1927 and 1928. Subsequently, the species was recovered by Mr. F. T. Scott of the State Department of Agriculture in August, 1928, near San Carlos, San Mateo County on Monterey pine, where it was feeding on *Kermes insignicola*. Later in July, 1934, specimens were taken in the same county at Redwood City on European sycamore infested with sycamore scale. The beetles were found under the loose bark on the trunks and in the curled terminal leaves. Nunenmacher (1934) reported the species as spreading to many parts of the state. Our observations of *E. quadripustulatus* feeding on the woolly apple aphid is probably the first recorded for this state, and the full importance of this ladybird beetle in controlling woolly apple aphid still remains to be determined. In central Europe it is considered to be a valuable predator of this pest. It certainly looks promising and was mainly responsible for reducing the aphid population in the orchard where the experimental plots were established in 1942 to a point where aphid colonies were difficult to find. No surveys were made in 1943, but on February 25, 1944, the orchard was

Table 1.—Percentage of mummified woolly apple aphids on twigs of apple in the Watsonville area, 1942, based on mature and nearly mature aphid.[1] The counts did not include the mummified aphids from which the parasites had previously emerged.

Orchard	Apple Variety	Mar. 27	Apr. 21	May 10	May 19	June 2	June 19	July 6	August 4	September 22
1[2]	Newton Pippin	6	21	10	8	25	21	37	27	3
2	Belleflower				9	19	23	36	41	13
3	Newton Pippin					22	23	14	—	—
4	Newton Pippin					1	1	—	17	—
5	Belleflower					3	16	33	23	45
6	Belleflower					2	3	—	—	—

[1] Where available 1,000 or more aphids were examined in each orchard on each survey.
[2] Inspections were made February 25, March 4, and March 19 in orchard number 1 at which time not over one per cent of the aphids were parasitized.

again examined. Aphid colonies were small and scarce, and many appeared as if they had at sometime been fed on by some predator. Adult ladybird beetles were found, as were recently laid eggs, which were probably those of *E. quadripustulatus*. Adult beetles were found in the only other orchard that was examined on the same date.

At the start of the investigation of the woolly apple aphid it was decided that the natural factors that might limit it should be studied. As a result, numerous surveys of apple orchards in the Watsonville area were conducted. Particular attention was paid to *Aphelinus mali*, a hymenopterous parasite, that had been established in the area since 1939. The parasite was introduced and became established through the cooperation of Mr. E. J. Newcomer at Yakima, Washington, and the Division of Beneficial Insect Investigation, University of California at Riverside. In 1942, the parasite was found rather widely spread throughout the Watsonville area. It was probably most abundant in one of the orchards where successful liberations were made in 1939. In determining parasitism, aphid colonies were collected at a number of points throughout an orchard. In determining the per cent of parasitism, only mature or nearly mature aphids were considered. These aphids and the dark mummified parasitized aphids were counted, and the per cent of mummified aphids determined by dividing the number of mummified aphids by the total number of aphids counted. In making counts, only the mummified aphids from which the parasites had not emerged were considered. The results of the surveys are given in table 1. Orchard number 1 is one of the locations where the parasite was successfully established in 1939. It is the only place where the parasite could be found in late winter. On February 25, one per cent of mummified aphids were found in this orchard. A number of other orchards were examined on this date but not enough aphid colonies could be found to determine whether any parasites were present. By March 27, the overwintering parasites had emerged and the first spring brood was making its appearance. Starting in June, the parasite could be found in most orchards, and parasitism in some was fairly high. The highest per cent of mummified aphids encountered during the season for any orchard on any particular survey ranged from about 40 to 45 per cent. During summer and fall, in heavily infested orchards where parasitism was high, nearly all aphid infested twigs and spurs were covered with the black mummies of parasitized aphids. Bell-flowers are apparently more heavily attacked by the woolly apple aphid than is the Newtown pippin, and as a result, the above was most noticeable in bellflower orchards. In such orchards, it would appear at a glance that the per cent of mummified aphids would be much higher than 40 to 45 per cent. However, a close examination of the twigs and spurs revealed that a very large proportion of the mummified aphids were those from which the parasite had already emerged and, therefore, represented parasitism of an earlier date.

Orchard number 4 represents the degree of parasitism found in the experimental orchard where *Exochomus quadripustulatus* was effective in reducing the woolly apple aphid population. In this orchard parasitism remained at a very low level although on August 4 the number of mummified aphids amounted to 17 per cent.

By mid-summer there was a marked reduction in aphid colonies in most orchards. For the most part the colonies were smaller, and on July 6 a number of twigs were observed where the aphid colonies had been completely destroyed by the parasite or predators or the two combined.

Aphelinus mali has probably not yet reached its full effectiveness in the Watsonville area. It should be further studied after it has had an opportunity to come to equilibrium with its new environment. On February 25, 1944, parasitism was checked in orchard number 1, and out of 207 woolly apple aphids 19 were mummified. The per cent of mummified aphids was 9.2 as against 0.9 on the same date in 1942.

In many orchards native predators were abundant and were doing much to reduce the woolly apple aphid population. Of these, syrphid fly larvae were the most numerous. The efficiency of the syrphid fly larvae is apparently reduced by a disease. In examining aphid colonies many dead larvae were encountered, and this was found to be the case in most orchards. Larvae of the green lacewing were rather abundant and must be a factor in helping to control the woolly apple aphid. On one or two occasions, adults of the ladybird beetle, *Coccinella californica*, were observed to be feeding on the pest.

Conclusions: Predators and parasites are important in helping to control the woolly apple aphid in the Watsonville area. In recent years two new beneficial insects have been established in the region by

the University of California. One of these is a ladybird beetle, *Exochomus quadripustulatus*, and the other is a hymenopterous parasite, *Aphelenus mali*. Both of these show much promise and their addition to the native beneficial insects is certainly desirable and may result, after equilibrium is reached, in satisfactory biological control of the woolly aphid.—7-5-44.

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The Power of the Analysis of Variance with the Poisson Distribution

WM. M. UPHOLT, *Orlando, Florida*

A recent article described a hypothetical experiment designed to determine certain properties of the statistical test known as the analysis of variance when the data used in the analysis follow a particular type of distribution. The results of this hypothetical experiment indicated that the technique in question was safe in reasonable cases in that it did not indicate a difference between means, when in fact no difference existed, with a frequency greater than that set by the level of significance chosen. On the other hand the evidence available indicated that under the conditions of the experiment the test had a rather low power in that it detected real differences of considerable magnitude with what was considered to be a rather low frequenty (Upholt 1942).

The particular technique described was not designed for use with contagious distributions; rather it was designed for use with data that follow a random distribution described by the Poisson law. Thus it is of interest to see how the technique behaves under the conditions for which it was designed.

Accordingly a hypothetical experiment was conducted following exactly the same procedure as that previously described except that it was assumed that the eggs of the insect were deposited singly instead of in masses. This one change means that the resulting larvae are all distributed over the fields independently and at random. Thus the treatments, which affected the distance travelled by the larvae at the time counts were made but did not kill any larvae, had no effect upon the frequency distributions and all three plots in any one block had identical distributions, described by the Poisson law, and with the variance equal to the mean. This describes the situation which would be expected with a nongregarious insect which lays its eggs singly and at random in a perfectly homogeneous field.

A randomized block experiment was constructed using the same means as previously. Random samples were selected by the use of random sampling numbers, the data were transformed by adding one half and extracting the square root and the transformed data were analyzed by the technique known as the analysis of variance. Values of F and of t were calculated for the difference between block means—means that were known to differ in actual fact by known amounts.

Table 1.—The power of tests when applied to the hypothetical experiment with a Poisson distribution.

	F-TEST	
RANGE OF ACTUAL MEANS	NO. OF CASES IN 50 SURPASSING 5% LEVEL OF SIGNIFICANCE	NO. OF CASES IN 50 SURPASSING 1% LEVEL OF SIGNIFICANCE
4 to 10	29 (=58%)	15 (=30%)
	t-TEST	
RATIO OF ACTUAL MEANS	t EXCEEDING THE 5% LEVEL OF SIGNIFICANCE	t EXCEEDING THE 1% LEVEL OF SIGNIFICANCE
8 to 10	4 (= 8%)	1 (= 2%)
4 to 10	33 (=66%)	9 (=18%)

Table 2.—The power of tests when applied to the hypothetical experiment with contagious distributions.[1]

	F- TEST	
RANGE OF ACTUAL MEANS	NO. OF CASES IN 100 SURPASSING 5% LEVEL OF SIGNIFICANCE	NO. OF CASES IN 100 SURPASSING 1% LEVEL OF SIGNIFICANCE
4 to 10	23 (=23%)	6 (=6%)
	t-TEST	
RATIO OF ACTUAL MEANS	t EXCEEDING 5% LEVEL	t EXCEEDING 1% LEVEL
8 to 10	0 (= 0%)	0 (=0%)
4 to 10	23 (=23%)	2 (=2%)

[1] Taken from Table 3, Upholt 1942.

Fifty such sets of samples and analyses were prepared. The number of t-values exceeding the 5 per cent and the 1 per cent levels of significance were counted for the two extreme comparisons. These results are presented in table 1 for comparison with the similar results obtained for contagious distributions as presented in table 2 (Upholt 1942). It must be born in mind that the figures for the data following a contagious distribution as presented in the previous paper are based upon one hundred cases whereas the figures in table 1 of the present paper are based upon only fifty cases. Equivalent percentages are put in parentheses following each figure to ease this comparison. When this fact is born in mind it is readily evident that this transformation and analysis has a much greater power to detect important differences when applied to data following a Poisson distribution, the case for which it was designed, than it does when applied to data following a contagious distribution such as might reasonably be expected with certain insects.—6-23-44.

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Taking Stock

DURING this very critical time in the life of our nation, we should all weigh with gravity the circumstances which have led to the catastrophe and more especially, concerning the future days that are to follow. The thinning out of our ranks and the transfer of additional duties and responsibilities upon those who are left at home, should awaken in us as well as in those who have gone abroad to fight the wars, an inspirational desire for the times that are to come. Those of us at home have had an opportunity to take stock and to see where we have been lax in the past. We have had an opportunity to examine our teaching, investigations, and extension work, and to see very clearly where this work might be substantially strengthened when reconstruction begins.

Those who have taken over the more hazardous duties of fighting the enemy will have formulated even clearer objectives for the future. It is not difficult to visualize how these young men sitting under the open skies in tropical and in arctic regions might ponder over the slender opportunities which they have had in the past, and determine that greater fields of service must lie before them when they return to their respective jobs and posts. I can also visualize what a tremendous disappointment and let-down would await these young men and women if they were to be dropped back into the very same positions that they left to enter the service of their country. It is for everyone of us now in charge of the entomological work at home to see that each of those who has served both at home and abroad is given adequate opportunities to build himself and the nation when the new peace arrives. There are many opportunities to be made and many things that need to be done before *we* are ready to take on these new projects. The members of the American Association of Economic Entomologists and those of the Entomological Society of America, and those of all other entomological clubs, societies, scientific academies, and similar organizations should unite in a common purpose towards this end. This work has already been started under the able leadership of such committees as the Committee on the Coordination of Entomology to the War Effort, the Committee on Postwar Planning, the Committee on Military Entomology and the entomological representations to the National Research Council.

It has been made plain in other issues of the JOURNAL that these groups are planning to collaborate in every possible way towards a joint, and unified effort to meet all of the various needs of entomologists after the war. It is needless to call attention to the fact that if we are to maintain professional standards, that all of the various fields in entomology must receive careful, just, and adequate consideration. In our haste to accomplish the most from an economic return, we have too often over-emphasized the wholly commercial side of our subject. Not that it should lack emphasis, but that it should become a part of the other essential fields in entomology. Basic training in a fundamental knowledge of insect taxonomy and insect biology, is the cornerstone of our profession. That cornerstone should be set out prominently so that every student who enters the field should not overlook its essentiality and importance. If I could write a message to all the teachers and instructors in entomology the world over, I would charge them with their duty and responsibility towards the development of a more thorough and more extensive program in teaching beginning students those basic principles which make the young man or young woman a thorough student of all phases of entomology. I fear that too often teachers have been giving more attention to the application of control measures before they have given the student a real knowledge of what is to be controlled. As an economic entomologist, I feel that I can talk plainly concerning the subject. And while I do thoroughly know that the control of insect pests are absolutely essential to the existence and comfort of the

human race and to our profession, yet I feel that such control cannot be consumated without a thorough scientific knowledge of insects and all their varying ecological implications.

It is too often evident that the more essential fundamentals of taxonomy and diagnosis of injurious insects has been neglected far beyond the comprehension of most workers in our field. Where are the old masters who were responsible for training the great group of entomological workers of our time? One by one these men have dropped out of our midst. Who are replacing the men of the broad training of J. H. Comstock, L. O. Howard, Herbert Osborn, H. T. Fernald, Lawrence Bruner, C. W. Woodworth, and numerous other men of their calibre? Who are replacing these men who were trained in the broad aspects of entomology—men who had the vision and the enthusiasm and the qualifications to inspire as well as to train men? We are the men who must take their places. Ours is a greater problem than that which faced these pioneers. Ours is the time of greater complexities in agriculture, in business, and in social life. To one problem that faced these men there are a hundred that face us.

Midst all of these rapidly developing uncertainties in our University curriculi and in the intricacies of new and more efficient and still more involved insecticides, it is not to be wondered that we have neglected some of those entomological ideals that were so fundamental to our forefathers and still appear as useful to all of us today.

In closing, I want to emphasize just two points in this article: First, the necessity of working out some program which will have as its chief consideration the placement and advancement of service men who return to their respective jobs in entomology; and second, that we must all very greatly improve our teaching curriculi so that the students of the future will be sufficiently well-trained to meet the multiplicity of problems that are sure to arise during the postwar period. This will mean very extensive broadening in practically every institution in the country.

E. O. ESSIG

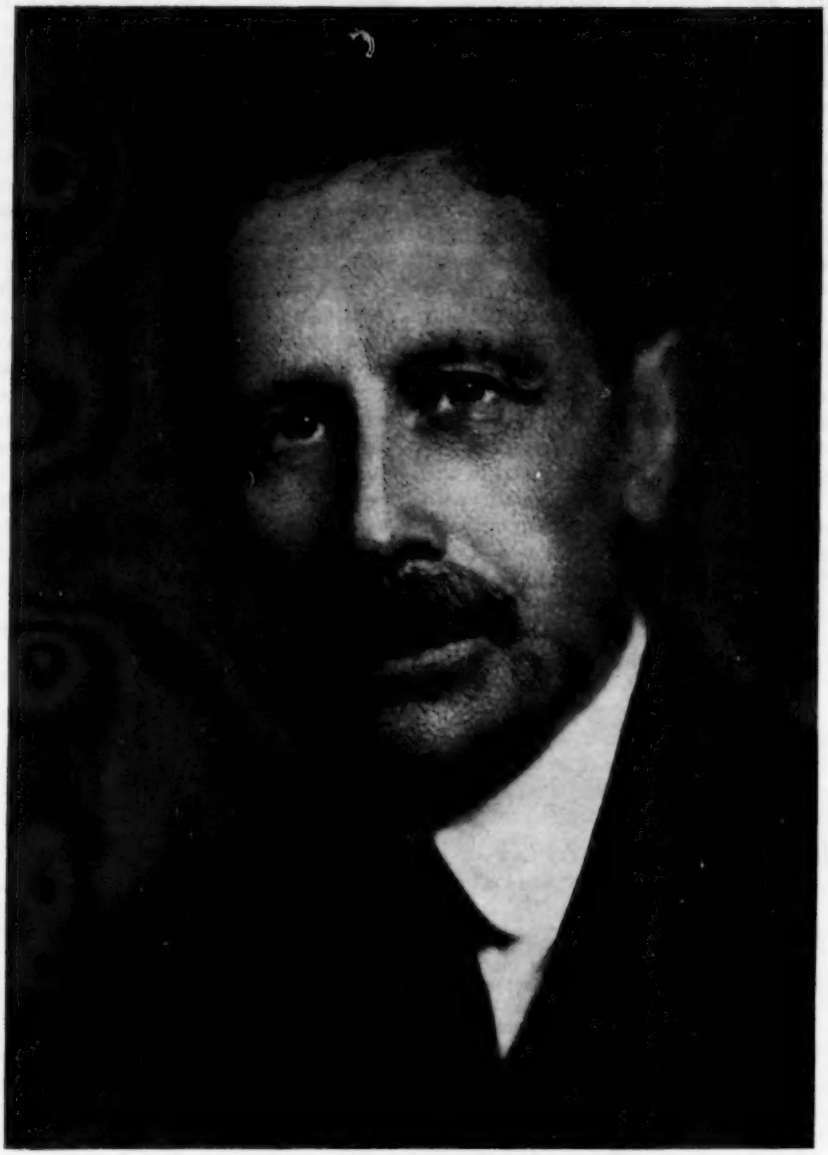
REVISIONS OF FEDERAL PLANT QUARANTINES AND ADMINISTRATIVE CIRCULARS

The Japanese beetle quarantine was revised effective March 30, 1944, to provide for more flexibility in the handling of seasonal and local problems through the issuance of administrative instructions by the Chief of the Bureau of Entomology and Plant Quarantine. The regulated areas were but slightly increased. Effective on the same date the Bureau issued circular B.E.P.Q. 533, listing the articles exempted from the restrictions of the quarantine as revised. Under circular B.E.P.Q. 535 dated June 2, the dates were set for beginning the seasonal restrictions in 1944 on the movement of cut flowers and fruits and vegetables from the heavily infested area interstate to points outside the regulated areas. June 12 were set as the beginning date for the southern section, and June 26 the northern section of that area.

GOLDEN NEMATODE SURVEY

A survey for the golden nematode, *Heterodera rostochiensis* Wollenweber, of potatoes was begun in June 1944 in the more important northern potato districts east of the Mississippi River, to determine as far as possible, the status of these districts with respect to this potato root parasite. First discovered in this country on Long Island, in 1941, the pest was reported to the National and Eastern Plant Boards at their annual meetings in October 1942, and the State of New York made a local survey in 1942 and placed a quarantine, effective March 13, 1944, on the movement of seed potatoes and soil from the affected area. The Bureau of Plant Industry of the U. S. Department of Agriculture cooperated in the survey. The current survey is being conducted by the Bureau of Entomology and Plant Quarantine in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering and appropriate agencies of the respective States.

OBITUARY



Harrison Garman, 1856-1944[2]

Harrison Garman[1] was born at Lena, Illinois on December 27, 1856 and died at Lexington, Kentucky, August 7, 1944. His early childhood was spent at Lena where he roamed through the fields and woods and became acquainted with nature at first hand. Later, the family moved to Normal, Illinois and settled close to the State Normal School in order to educate the children. There he learned how to teach under Thomas Metcalf and came in contact with S. A. Forbes and his associates of the Illinois State Laboratory of Natural History. A year was spent in study at Johns Hopkins University under the guidance of Professor Brooks. When the State Entomologist's office was transferred to Champaign in 1883, he went with it, becoming first assistant to the State Entomologist and Associate Professor of Zoology at the University. In 1889 he became head of the Department of Entomology and Botany of the Kentucky Agricultural Experiment Station and was made State Entomologist in 1897. He was appointed Professor of Entomology

[1] Originally William Harrison; the William was dropped about 1881.
[2] Photograph dated 1929.

and Zoology at the University of Kentucky in 1912. During his term of office as Entomologist and Botanist at the Kentucky Station, he was instrumental in securing the enactment of the first state nursery inspection law, as well as a pure seed law which is still in operation. He built up in his department a substantial collection of insects and a herbarium of plants which formed the basis of much of his scientific work.

Professor Garman published, in all, about 170 papers, bulletins or scientific articles. Of these, 99 were on entomology, 58 dealt with botanical subjects and 18 were on zoological topics other than entomology. A number of these dealt with both botany and entomology. At the time of his retirement he had projected a general work on the insects which he was not able to finish. His interest in Natural History never failed and at the time of his death the home lot was planted with many of the wild flowers, trees and shrubs which he loved so much.

At one time or another Professor Garman was a member of 16 scientific societies including the American Association of Economic Entomologists, serving as secretary in 1893 and president in 1906. He was elected to fellowship in the American Association for the Advancement of Science in 1922. The University of Kentucky granted him the degree of Doctor of Science in 1912.

Professor Garman married Rosalie Miller of Hatfield, Massachusetts, in 1883 and is survived by two sons, Frederick, of Juniata, Pa., and Philip, of Hamden, Connecticut.—P. G.

DR. E. D. SANDERSON

Dr. Dwight Sanderson, better known among entomologists as E. D. Sanderson, died on September 27 in Ithaca, New York. Dr. Sanderson suffered a stroke on a journey from Florida back to his home in Ithaca and died soon after arrival. He served as President of our Association in 1910. An obituary notice will follow.

DR. E. W. BERGER

Science has announced the death, on August 23, of Dr. E. W. Berger, Entomologist of the Florida State Plant Board until his retirement in 1943. Dr. Berger was long a member of our Association and we shall hope to secure an obituary article for later publication.

PAUL KNIGHT RETURNS

Paul Knight, formerly on the staff of the Department of Entomology, University of Maryland, and since February, 1943 working in Latin America, returned to this country June 30, 1944. Mr. Knight was employed by the Food Supply Division, Office of the Coordinator of Inter-American Affairs for the purpose of assisting in establishing emergency food missions in four Central American countries and Panama, with headquarters in San José, Costa Rica. Before returning to Washington he spent two months in Perú where he assisted the Servicio Cooperative Inter-Americano de Produccion Alimentos, of Lima, a joint project of the Ministerio de Agricultura del Perú and the Coordinator of Inter-American Affairs. In October, 1944, Mr. Knight joined the staff of the Washington, D. C., office of the United Nations Relief and Rehabilitation Administration. He may now be addressed at 1344 Connecticut Avenue, Washington, 25.

DR. STEWART RETURNS FROM BOLIVIA

Dr. M. A. Stewart has recently returned to the University of California to resume his duties at that institution after having spent several months as a consulting parasitologist to the Government of Brazil. Part of Dr. Stewart's time was devoted to travel to inspect the conditions prevailing in the country and a part to the establishment of laboratories. Headquarters for the work were at Santa Cruz, seat of the institutions most concerned with his work.

Some time was devoted to Rio de Janeiro and San Paula, in Brazil and Dr. Stewart also visited Perú where he attended conferences dealing with the plague.

No doubt Dr. Stewart's experience will be reflected in his future work in this country and we feel confident, also, that he was not only a valuable counselor to the scientists in the countries he visited but also an effective promoter of good will with those countries.